

Role of Focal Adhesion Kinase in the invasive phenotype of small-cell lung cancer

Frank ABOUBAKAR NANA

Thesis submitted to the Faculty of Biomedical Sciences of Université catholique de Louvain in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Biomedical and Pharmaceutical Sciences

Private defense on April 1st 2020

Promotor:

Professor Sebahat OCAK, UCLouvain, Secteur des Sciences de la Santé, Institut de Recherche Expérimentale et Clinique (IREC), Pole of Pneumology, ENT and Dermatology (PNEU) & CHU UCLouvain Namur (Godinne Site), Division of Pneumology, Belgium

Co-promotor:

Professor Charles PILETTE, UCLouvain, Secteur des Sciences de la Santé, IREC, PNEU & Cliniques universitaires St-Luc, Division of Pneumology, Belgium

MEMBERS OF THE JURY

President:

Professor Jean-Pascal MACHIELS, UCLouvain, Secteur des Sciences de la Santé, IREC, Molecular Imaging, Radiotherapy and Oncology (MIRO) & Cliniques universitaires St-Luc, Division of Medical Oncology, Belgium

Members:

Professor Anabelle DECOTTIGNIES, UCLouvain, Genetic and Epigenetic Alterations of Genomes, de Duve Institute, Faculty of Pharmacy and Biomedical Sciences, Belgium

Professor Olivier FERON, UCLouvain, IREC, Pole of Pharmacology and Therapeutics (FATH), Faculty of Pharmacy and Biomedical Sciences, Belgium

Invited members:

Professor David PLANCHARD, Université Paris-Saclay, Department of Medical Oncology, Gustave Roussy, Villejuif, France

Professor Karim VERMAELEN, Universiteit Ghent, Department of Pulmonary Medicine - Tumor Immunology Laboratory, -Belgium

Acknowledgments

« J'ai répondu qu'on ne changeait jamais de vie, qu'en tout cas toutes se valaient et que la mienne ici ne me déplaisait pas du tout ». L'étranger de Albert Camus (1942).

Madame Wollanders à qui j'exprime ma grande gratitude pour m'avoir accompagné tout au long de ma thèse sur le plan organisationnel et administratif avec une réactivité, une disponibilité et une efficacité hors norme.

Merci aux membres du comité d'accompagnement et aux membres du jury pour leur revue sans complaisance, pointilleuse et constructive qui donne une valeur précieuse à ce travail.

Au Professeur Yves Sibille

Merci d'avoir décelé, initié et partagé avec moi cette idée de la recherche fondamentale comme arme supplémentaire aux soins apportés aux patients dans notre pratique médicale. Vous avez su m'orienter vers les bonnes personnes avec lesquelles j'ai pu mener à bien ce projet. Mon seul petit regret, c'est de ne pas avoir eu l'opportunité de défendre cette thèse avant votre éméritat.

Au Professeur Sebahat Ocak

Chère Sebahat, merci pour l'honneur que vous m'avez accordé en me prenant sous votre aile et en acceptant de diriger ma thèse. Je vous serai toujours reconnaissant de votre bienveillance, votre sens aigu du détail, vos critiques constructives et le respect que vous m'accordez sur chacun de nos échanges. Veuillez trouver l'expression de mon profond respect, ma grande estime et mes sincères remerciements.

Au Professeur Charles Pilette

Cher Charles, votre calme britannique, votre sens aigu du détail, votre humour et votre grande sagesse ainsi que vos conseils précieux m'ont tout de suite donné un sentiment de sécurité et de protection dans votre laboratoire. Le soutien indéfectible que vous témoignez au laboratoire s'est poursuivi jusqu'en clinique où vous avez été le plus grand artisan pour que je puisse rejoindre l'équipe de pneumologie clinique. Ceci sans doute en raison de votre thème de prédilection : la recherche translationnelle dont nous partageons l'intérêt et que j'ai eu l'occasion de parfaire dans l'une des plus grandes équipes au monde en la matière grâce à l'aide de Sebahat. Mes sincères remerciements.

Cette période au laboratoire a surtout été une opportunité de rencontrer des personnes formidables sans lesquelles ce travail n'aurait sans doute jamais vu le jour. Cela a aussi rendu cette période de ma vie enrichissante. Seul le plus grand auteur contemporain et prix Nobel de littérature l'exprime le mieux dans « Ville triste » (1975) : « Il y a des êtres mystérieux, toujours les mêmes, qui se tiennent en sentinelles à chaque carrefour de notre vie. » Patrick Modiano.

Marylène, avec qui je partage ce travail grâce à son soutien émotionnel, psychologique, et sur les travaux de recherche. Tu ne m'as jamais lâché même dans mes idées absurdes. Nous avons même réussi à réanimer des membranes de western blot rongées par l'acide. Mille mercis et du fond du cœur. J'ai eu de la chance de t'avoir rencontré.

Merci aux conseils de Bruno, Maha et Sébastien. Je remercie la douce et adorable Amandine, l'espiègle Diana. Mes voisins de bureau successifs John, François qui ne sont pas aussi difficiles à vivre que moi. Je n'oublie pas Charlotte et sa simplicité, Thomas et ses milliards de neurones effervescents et sa sympathie. Marie, sans doute la plus intelligente d'entre nous. Je remercie aussi mes compères du laboratoire et en clinique, Antoine, Sophie et Ludovic

Je ne remercierai jamais assez Caroline, Chantal, Michèle de la plateforme 2IP qui m'ont suivi dans tous mes projets et apporté de l'aide dans toutes mes idées folles, irréalisables au départ mais accomplies finalement. Je remercie aussi Isabelle, Philippe, Anne-Thérèse V., Anne-Thérèse P., Najet, Anne, Mélanie, François, Eléonore, Johan, Kate, Fairouz.

Merci à Jérôme pour ton aide précieuse, ta disponibilité et ta gentillesse. A nos prochaines collaborations. Mes remerciements à Bertrand de la plateforme du CTMA, à Nicolas et Aurélie qui m'ont été d'une aide précieuse sur le développement du marquage multiplex en immunofluorescence.

Je remercie tous mes collègues de l'équipe de pneumologie qui m'ont accueilli en clinique, le chef de service T. Pieters, mes supporters et soutiens : Françoise, Antoine, Giuseppe, Benny. Mon voisin et confident et sage Philippe. Ma collègue de bureau Sophie. Merci aux infirmières du plateau technique, Mélanie, Bahiya, Brigitte, Laurence pour leur accessibilité, leur compétence et leur souci des soins apportés aux patients dans le contexte particulier et stressant des techniques pneumologiques. J'admire votre dévouement et vos qualités humaines. Vanessa, Irina, Sandra regroupées dans le bureau de « la bienveillance », accueillant tout en gardant votre bonne humeur. A Sylvie notre coordinatrice de soins en oncologie thoracique et à nos infirmières de l'unité d'hospitalisation.

A toute l'équipe de fonction respiratoire, Zakia, Joachim, Jesus, Cécile. A toute notre équipe du secrétariat, Catherine, Alexia, Jennifer et surtout Anne-Sophie qui est toujours là pour m'apporter son aide sans compter, même pendant ses congés. Merci à l'équipe de kiné : Ines et surtout à mon ami Gregory pour son énorme cœur sur la main. Merci pour ta relecture et ton regard aiguisé sur mon manuscrit.

Merci à Valérie, mon binôme en oncologie thoracique qui m'a tout de suite accueilli dès le premier jour et qui me témoigne sa confiance et son profond respect. Tu es une femme exceptionnelle, et une chirurgienne hors pair que j'admire sincèrement. Delphine, merci pour ta disponibilité, ton écoute, ta bienveillance envers moi malgré les 100 coups de fils par jour. Merci à Benoit et Nadia pour nos échanges et votre expertise. Grâce à vous quatre, ma vie dans l'institution est plus aisée et mes projets de recherche ont pu se poursuivre.

Je ne peux terminer cet exercice de mémoire sans remercier ma deuxième famille professionnelle où j'ai appris ce métier dans des conditions exceptionnelles dans l'une des meilleures équipes qui pratique de la médecine de précision au monde tout

en préservant l'humanité qui est indispensable quand on s'occupe du cancer le plus mortel. Merci à Benjamin et David de m'avoir réservé un accueil singulier et intégré dans votre équipe remarquable dès mon premier jour. Merci de continuer à m'aider à grandir. Merci à Laura, Thierry, Anas, Laetitia, Céline, Marie et toute l'équipe du comité 031 de Gustave Roussy et du centre chirurgical Marie-Lannelongue.

Merci à mes collègues du service de pneumologie de CHU UCLouvain Namur site de Godinne, plus particulièrement Lionel Pirard et Fabrice Duplaquet pour leur aide pour nos projets de recherche. Il en est de même pour l'équipe de biobanque du CHU UCLouvain Namur site de Godinne qui m'a énormément aidé durant toute ma thèse et qui continue à être disponible pour moi en y mettant tout son cœur : Fabienne, Laurence, Sophie et Laure.

Je remercie aussi l'équipe de pneumologie de l'hôpital Saint Luc Bouge qui a accompagné mes premiers pas de médecin avec beaucoup de bienveillance. Merci particulier à Richard Frogner, Pierre Bachez et Pascal Legros.

Remerciements personnels

A mes parents, mes sœurs et ma partenaire de vie

Merci pour votre amour inconditionnel. Maman, à ta force de caractère, ta bienveillance et ton courage. Grâce à toi je suis devenu la personne authentique que je suis aujourd'hui. A Mireille, ma deuxième maman. Pour tes encouragements ton soutien moral, financier durant ces interminables années de médecine puis cette thèse de sciences qui voit enfin le jour. Merci à toi et Hugo pour les déménagements de kot à chaque début et fin d'année. A Germaine, Sandrine : mon plus grand bonheur est de voir dans votre regard l'estime et la fierté que vous me témoignez.

A mon père et mon petit frère, les figures masculines de ma vie dont les souvenirs heureux résonnent et demeurent intacts comme « une madeleine de Proust » comme si c'était hier. A Amélie, mon âme sœur, la grande source de joie de mon existence qui partage mes doutes au quotidien. Tu es la personne qui a le plus subi cette thèse à cause de mes longues journées, mes weekends passés au laboratoire, à venir me chercher au laboratoire à 23h après ta journée de travail, même en étant enceinte. J'espère que notre fils qui est plus jeune que cette thèse apprenne de ta formidable personnalité et de ton intelligence.

A mes amis, mes frères Fabien et Boris, des merveilleuses rencontres depuis les débuts de nos études. Votre simplicité et le souci des autres est sans doute la chose que nous avons en commun. A mes neveux et nièces ainsi qu'à mes filleuls Bram et Raphaël.

ABBREVIATIONS

ADC, adenocarcinoma

Ago2, Argonaute 2

Akt, AKT Serine/Threonine Kinase

ALDH1, aldehyde dehydrogenase 1 family

ALK, anaplastic lymphoma kinase

APC, adenomatous polyposis coli

ASCL1, achaete-scute homologue 1

ATM , ataxia telangiectasia mutated

ATR, ataxia Telangiectasia and Rad3-related

BRAF, v-Raf murine sarcoma viral oncogene homologue

BrdU , bromodeoxyuridine

BRS-3, bombesin receptor subtype 3

CCL1, chemokine (C-C motif) ligand 1

Ccl1, chemokine (C-C motif) ligand 1 gene

CCL5, chemokine (C-C motif) ligand 5

Ccl5, chemokine (C-C motif) ligand 5 gene

CDK: Cyclin-dependent kinases 1, 2,3

CDKN2A, cyclin dependent kinase inhibitor 2A

CGH, comparative genomic hybridization

CGRP, calcitonin gene-related peptide

CHK2, checkpoint kinase 2

c-IAP, cellular inhibitor of apoptosis protein 1

CSC, cancer stem cells

CTLA-4, cytotoxic T lymphocyte-associated protein 4

DAB, diaminobenzidine

DAPK, death-associated protein kinase

DCIS, ductal carcinoma in situ

DDR, DNA damage repair

DMSO, dimethyl sulfoxide

ECM, extracellular matrix

ED, extensive disease

EGF, epidermal growth factor

EGFR, epidermal growth factor receptor

FAK, focal adhesion kinase

FAT, focal adhesion targeting

FERM, Protein4.1-ezrin-radixin-moesin ()

FFPE, formalin-fixed paraffin-embedded

FGF, fibroblast growth factors

FGFR1, fibroblast growth factor receptor 1

FHIT, fragile histidine triad diadenosine triphosphatase

FISH, fluorescent in situ hybridization

FRNK, FAK-related non-kinase

GADD45, growth arrest and DNA damage-inducible 45

GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Grb7, growth factor receptor bound protein 7

GRP, gastrin-releasing peptide

GRPR, gastrin-releasing peptide receptor

GSTP1, glutathione-S-transferase P1

HER2 (also known as ERBB2), human epidermal growth factor receptor 2

HGF, hepatocyte growth factor

HGNECs, high-grade neuroendocrine carcinomas

HNSCC, head and neck squamous cell carcinoma

HRP, horseradish peroxidase

ICI, immune check-point inhibitor

IFCT, Intergroupe Francophone de Cancérologie Thoracique

IGF, insulinlike growth factor

IGF-IR, insulin-like growth factor 1 (IGF-1) receptor

IHC, immunohistochemistry

IL-16, interleukin 16

IL-1RAcP, interleukin-1 receptor accessory protein

IL-1 α , interleukin 1 alpha

IL-2, interleukin 2

IL-4 interleukin 4

IL-6, interleukin 6

IL-33, interleukin 33

IRS2, insulin receptor substrate 2

JNK1, c-Jun N-terminal kinases 1

KIT, KIT proto-oncogene (also known as CD117)

KLF8, Kruppel like factor 8

KRAS, Kirsten rat sarcoma viral oncogene homologue

LCNECs, large-cell neuroendocrine carcinomas

LD, limited disease

LDCT, low-dose CT

LOH, loss of heterozygosity

MDSC, myeloid-derived suppressor cells

MEK, also known as Mitogen-activated protein kinase kinase (MAP2K)

MET, hepatocyte growth factor receptor

mIF-IHC, multiplex immunofluorescence immunohistochemistry

MLL2, myeloid/lymphoid or mixed-lineage leukemia 2

MMP2, metalloproteinases 2

MMP9, metalloproteinases 9

MYC, myelocytomatosis viral oncogene homolog

NeuroD1, neurogenic differentiation factor 1

NF- κ B, nuclear factor-kappa B

NGS, next-generation sequencing

NLST, National Lung Screening Trial

NMB, neuromedinB

NMBR, neuromedin B receptor

NSCLC, non-small cell lung cancer

NTRK, neurotrophic tropomyosin receptor kinase

Oct3/4 octamer-binding transcription factor 3/4

OS, overall survival

p130 CAS, p130 Crk-associated substrate

p190RhoGEF, p190 Rho guanine nucleotide exchange factors

PAHs, polycyclic aromatic hydrocarbons

PARP, poly (ADP-ribose) polymerase

PD-1, programmed cell death 1

PDGR, platelet-derived growth factor

PD-L1, programmed cell death ligand 1

PFS, progression free survival

PI3K, phosphatidylinositol 3-kinase

PIK3CA: phosphatidylinositol-3-kinase, catalytic subunit α

PIK3R2, phosphatidylinositol 3-kinase regulatory subunit beta

PNETs, pulmonary neuroendocrine tumours

POU2F3, POU class 2 homeobox 3

PRRs, proline-rich regions

PTEN, phosphatase and tensin homolog

Pyk2, proline-rich tyrosine kinase 2

Rac1, Ras-related C3 botulinum toxin substrate 1

RAK, RAK tyrosine kinase (also known as Tyrosine-protein kinase FRK)

RB, retinoblastoma gene

RET, ret proto-oncogene

RFS, recurrence free survival

ROS, reactive oxygen species

ROS1, ROS proto-oncogene 1

RTKs, receptor tyrosine kinases

SCC, squamous cell carcinoma

SCLC, small cell lung cancer

shRNA, short/small hairpin RNA

SOX2, sex determining region Y box 2

ST2L, longer membrane bound form

STK11, serine/threonine kinase 11 (also called LKB1)

TAM, tumor-associated

TCGA, The Cancer Genome Atlas

TGF- β , transforming growth factor beta

TMA, tissue microarrays

TP73, tumor protein p73

Treg , regulatory T cells

TSA, tyramide signal amplification

TSG, tumor suppressor gene

UGT1A1, UDP-glucuronosyltransferase 1-1

VEGF, vascular endothelial growth factor

VEGF, vascular epithelial growth factor

VEGFR-3, vascular endothelial growth factor receptor 3

WB, western blot

XIAP, X-linked inhibitor of apoptosis protein

YAP1, yes-associated protein 1

1	INTRODUCTION	16
1.1	Lung cancer epidemiology	16
1.2	Lung cancer etiology	17
1.3	Lung cancer classification	19
1.4	Lung cancer treatment and prognosis	21
1.4.1	Non-small-cell lung cancer	22
1.4.2	Small-cell lung cancer	24
1.4.2.1	Treatment options in first-line setting of small-cell lung cancer ..	24
1.4.2.2	Second-line and subsequent therapy of small-cell lung cancer ..	25
1.4.2.3	Targeting angiogenesis in Small-cell lung cancer	27
1.5	Towards a new molecular classification of small-cell lung cancer	30
1.6	Role of focal adhesion kinase in small-cell lung cancer and its potential as a therapeutic target	39
1.6.1	Introduction	40
1.6.2	FAK overexpression and/or activation in human cancers, its frequency and mechanisms	45
1.6.3	FAK role in proliferation, cell cycle, and survival	48
1.6.4	FAK role according to cellular compartment	49
1.6.5	FAK role in adhesion, migration, and invasion	52
1.6.6	FAK in epithelial to mesenchymal transition	53
1.6.7	FAK-mediated angiogenesis and vascular permeability	54
1.6.8	FAK and DNA damage repair	57
1.6.9	FAK and radioresistance	58
1.6.10	Regulation of cancer stem cells	59
1.6.11	FAK in tumor immune escape	60
1.6.12	Interplay between FAK and Rho-family GTPases at the focal adhesion complex	62
1.6.13	Prognostic and predictive value of FAK alterations	63
1.6.14	Conclusions and therapeutic perspectives	64
2	BACKGROUND AND GOALS OF THE THESIS RESEARCH	66
3	INCREASED EXPRESSION AND ACTIVATION OF FAK IN SMALL-CELL LUNG CANCER COMPARED TO NON-SMALL-CELL LUNG CANCER	68
3.1	Abstract	68
3.2	Introduction	69
3.3	Materials and methods	71

3.3.1	Patients and tissues samples.....	71
3.3.2	Multiplex immunofluorescence immunohistochemistry (mIF-IHC) ..	71
3.3.3	Stained slides imaging	73
3.3.4	Quantitative evaluation of immunostaining	73
3.3.5	Western blot	77
3.3.6	Statistical analysis	77
3.4	Results.....	78
3.4.1	Patient Characteristics	78
3.4.2	FAK expression and activity are higher in SCLC than NSCLC and normal lung.....	81
3.4.3	FAK expression and activity do not correlate with patient characteristics or survival.....	86
3.5	Discussion	88
3.6	Conclusion	91
4	THERAPEUTIC POTENTIAL OF FOCAL ADHESION KINASE INHIBITION IN SMALL CELL LUNG CANCER	92
4.1	Abstract.....	93
4.2	Introduction	94
4.3	Materials and methods.....	95
4.3.1	Cell culture	95
4.3.2	Drugs.....	95
4.3.3	Lentivirus construction	96
4.3.4	Lentivirus production and cell lines' transduction with FAK shRNA and/or FRNK	96
4.3.5	Western blot (WB).....	97
4.3.6	Cell proliferation	97
4.3.7	Cell cycle analysis	97
4.3.8	Apoptosis assay	98
4.3.9	Wound healing assay associated with time-lapse video recording of cell motility.....	98
4.3.10	Matrigel invasion assay	98
4.3.11	Rac pull-down assay for activated GTPases	99
4.3.12	Statistics	99
4.4	Results.....	99
4.4.1	Pharmacological inhibition of FAK has several anti-tumoral effects in SCLC	99
4.4.1.1	PF-228 inhibits FAK phosphorylation at Tyr397	99
4.4.1.2	PF-228 inhibits proliferation and progression through cell cycle in SCLC	100
4.4.1.3	PF-228 induces apoptosis in SCLC	101
4.4.1.4	PF-228 inhibits migration and invasion in SCLC.....	101

4.4.1.5	Inh14 and PF-271 also inhibit proliferation and induce apoptosis in SCLC	104
4.4.2	Genetic inhibition of FAK leads to anti-tumoral effects only in presence of FRNK.....	105
4.4.2.1	Loss of total FAK following FAK shRNA transduction does not affect proliferation and progression through cell cycle in SCLC	105
4.4.2.2	FRNK overexpression following transduction inhibits proliferation and survival in SCLC.....	107
4.4.2.3	FRNK overexpression following transduction in SCLC cell lines previously transduced with FAK shRNA inhibits proliferation and survival	110
4.4.2.4	FRNK keeps Rac1 GTPase inactivated in SCLC	112
4.5	Discussion	114
5	CONCLUSIONS, LIMITATIONS AND FUTURE PERSPECTIVES	119
5.1	Conclusions	119
5.2	Limitations.....	120
5.3	Future perspectives	121
6	SUPPLEMENTARY DATA	124
7	FIGURES	127
8	TABLES	134
9	LIST OF PUBLICATIONS, ABSTRACTS, ORAL PRESENTATIONS, GRANTS, AND HONORS RELATED TO THE THESIS WORK	135
9.1	Publications	135
9.2	Astracts.....	135
9.3	Peer-reviewed oral presentations	136
9.4	Grants	136
10	REFERENCES	138

1 INTRODUCTION

1.1 Lung cancer epidemiology

Lung cancer is the most common cancer in the world, with about 2.1 million new cases diagnosed in 2018 in both sexes. It represents 11.6% of all new cancer diagnoses worldwide in front of breast, colorectum, and prostate cancers which account for 11.6%, 10.2%, and 7.1%, respectively [1] (**Fig. 1**).

Due to its high mortality rate, lung cancer is also the leading cause of cancer-related mortality in the world in both sexes with about 1.7 million deaths in 2018. It accounts for 18.4% of cancer-related mortality in both sexes ahead of colorectum, stomach, liver, and breast cancers which represent 9.2%, 8.2%, 8.2%, and 6.6% cases (fig.1.2), respectively [1] (**Fig. 2**).

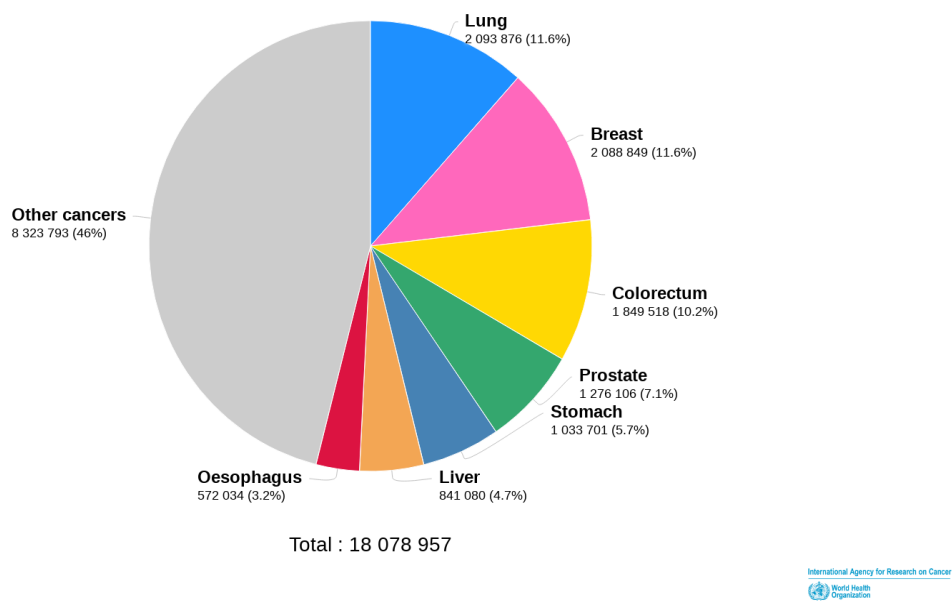


Figure 1: Estimated global numbers of new cancer cases with proportions worldwide, both sexes combined, in 2018. The area of the pie is proportional to the number of new cases (Globocan 2018 – <http://gco.iarc.fr>).

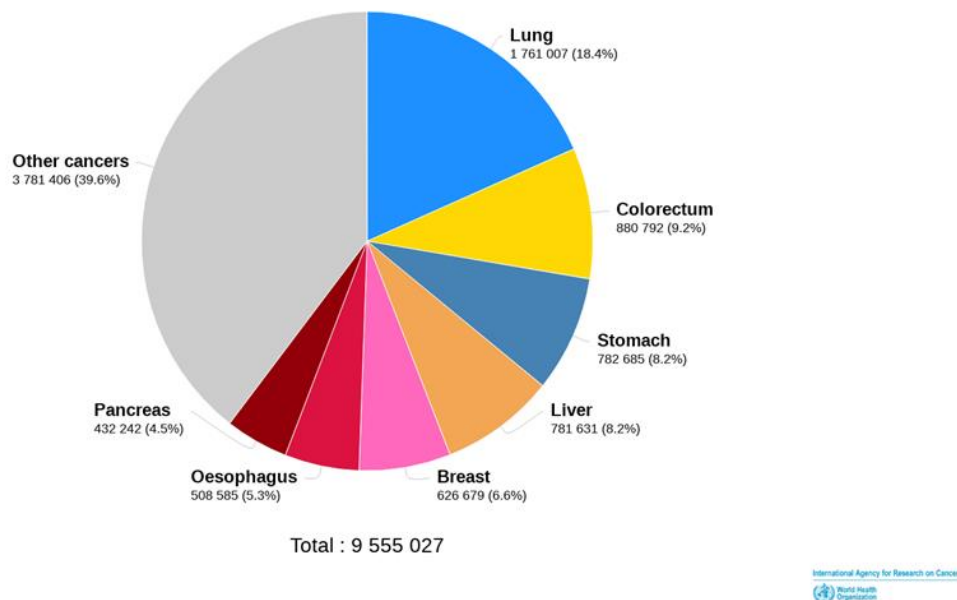


Figure 2: Estimated global numbers of cancer-related deaths with proportions worldwide, both sexes combined, in 2018. The area of the pie is proportional to the number of deaths (Globocan 2018 – <http://gco.iarc.fr>).

1.2 Lung cancer etiology

Tobacco smoking is well known as the leading and preventable cause of lung cancer. The risk for lung cancer increases with packs smoked per day, smoking duration, the age at first cigarette exposure, and total smoke exposure (pack-years) [2-5]. Cigarette as well as cigar or pipe smoking increases the risk of developing and dying from cancer in general, including lung cancer [2-6]. Male smokers lose about ten and female smokers about 11 years of lifespan [2]. It has been shown that smoking cessation decreases the risk of developing lung cancer as well as the lung cancer-related mortality and overall mortality [2,4,5]. Cigarette smoke is a complex aerosol composed of gaseous and particulate compounds that contains many potential carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aromatic amines, N-nitrosamines, and other organic and inorganic compounds, such as benzene, vinyl chloride, arsenic, and chromium. Cigarette smoke deposits across the whole bronchial tree and exerts carcinogenicity through more than 60 chemicals which bind to and chemically modify DNA (mutations) [7,8] and potentially induce carcinogenesis [9].

Other non-smoking-related factors such as residential radon exposure, professional asbestos exposure, and air pollution [10-14] have also been established as risk factors for lung cancer. These non-smoking-related risk factors may explain pathogenesis of some lung cancers arising in never smokers, which account for 5 to 25% of lung cancers. Residential radon exposure is considered as the second environmental cause of lung cancer in the whole population and as the first in never smokers [15-17] .

The interactions between tobacco smoking and other non-smoking-related risk factors such as residential radon exposure or asbestos substantially higher the risk of developing lung cancer (**Fig. 3**) as well as the lung cancer-related death [10,11,13].

The overwhelming cause of small-cell lung cancer (SCLC) in particular is cigarette smoking, the risk increasing with early age at initiation, intensity, and duration. Almost all SCLC cases occur in heavy and longtime smokers [45]. Smoking cessation results in rapid and substantial reduction risk for SCLC [45]. All lung subtypes have been found to be associated radon exposure, but high radon exposure tended to increase the risk of SCLC (**Fig. 4**) [10].

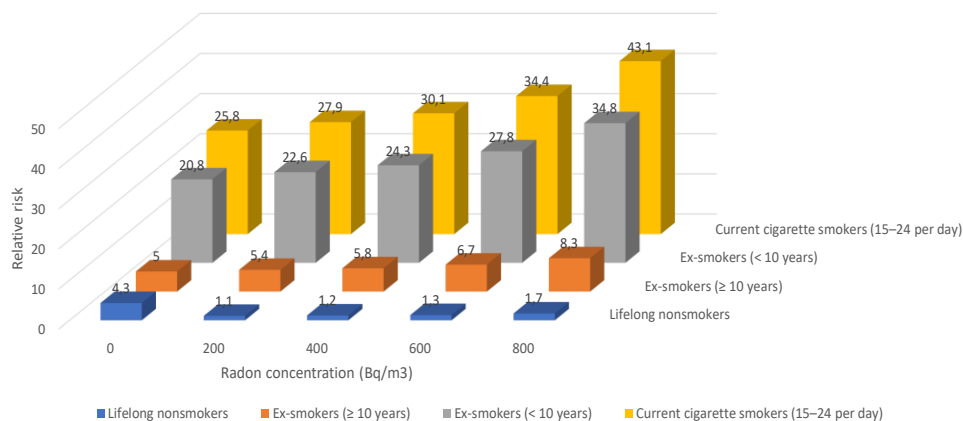


Figure 3: Combined estimates of relative risk for lung cancer according to smoking status and radon concentration exposure.

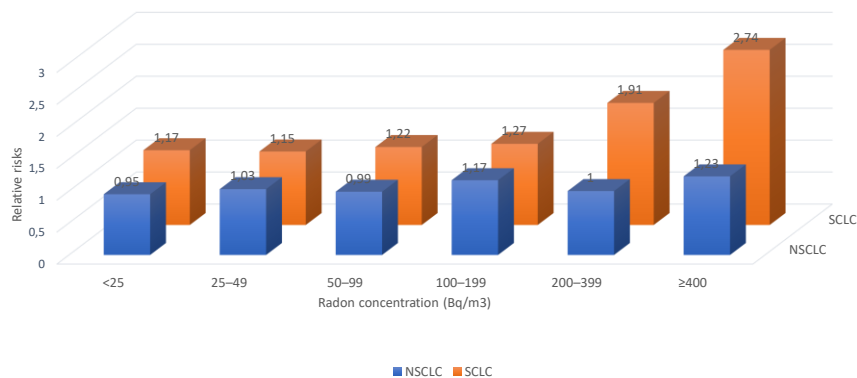


Figure 4: Relative risk of major histological types of lung cancer according to residential radon concentration exposure.

1.3 Lung cancer classification

Lung cancer is a clinically, histologically, and molecularly heterogeneous disease. Histologically, lung cancer, which arises from lung epithelial cells, is divided into two main types: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC is divided into three histological subtypes: adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large cell carcinoma. NSCLC and SCLC account for approximately 85% and 15% of all lung cancers, respectively [18]. Each histological (sub)type of lung cancer arises in different anatomical compartments of the lung airway epithelium. Alterations occurring in the basal cells or neuroendocrine cells located either in the central airway compartment or the bronchioles predominantly generate SCC or SCLC histology, respectively, while alterations in type 2 pneumocytes or club cells located in the peripheral airway compartment predominantly generate ADC [19] (**Fig. 5**). Clinically, SCLC is the most aggressive type of lung cancer, characterized by rapid doubling time, high growth fraction, a tendency for early dissemination, and a median five-year overall survival of 5% only [20,21].

Treatment decisions in metastatic lung cancer were exclusively based on histological types for several decades until recently, NSCLC and SCLC being treated with different chemotherapy regimens but all NSCLC subtypes treated similarly despite very different molecular features. This homogeneous approach to a heterogeneous disease contributed to the absence of substantial outcome improvements for many years [22].

Over the last 30 years, advances in next-generation sequencing (NGS) technology have led to vast improvements in our understanding of the molecular biology that underpins NSCLC, including identification of key and potentially targetable genetic aberrations. A molecular classification of NSCLC has emerged, besides histological classification, leading to new biological insights and targeted therapies directed towards specific molecular abnormalities found in small subsets of NSCLC, such as mutations in epidermal growth factor receptor (EGFR) genes or translocated anaplastic lymphoma kinase (ALK), RET, or ROS1 genes [23,24] (**Fig. 6**).

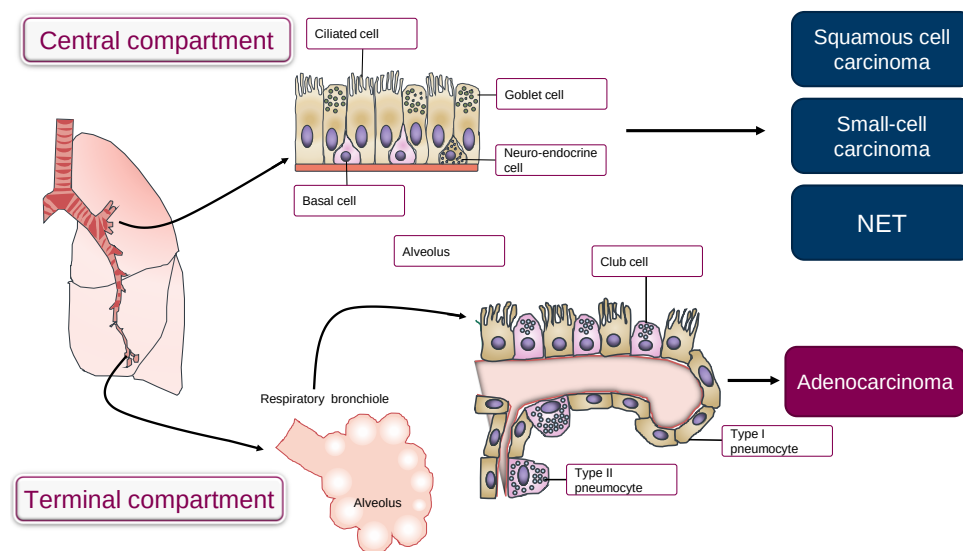


Figure 5: Schematic showing the location of the different cell types constituting the epithelial surfaces of the airways. Basal cells of the submucosal glands as well as central compartment are suggested to give rise to squamous cell carcinomas. Cells from peripheral airway compartment (type 2 pneumocyte) give rise to adenocarcinomas. Small-cell lung cancers and neuroendocrine tumors (NETs) arise from neuroendocrine cells located both in proximal compartment and submucosal glands.

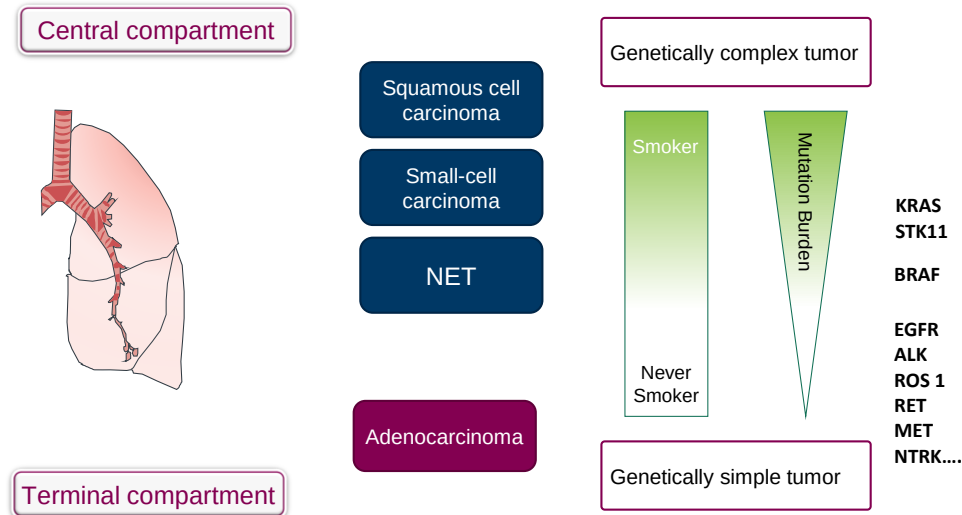


Figure 6: Molecular classification of lung cancer. In non-small-cell lung cancer, previously classified by histological features only, a molecular classification has recently emerged, which determines tumor behavior and response to therapy. Small-cell lung carcinomas, squamous cell carcinomas, and adenocarcinomas harboring KRAS and STK11 mutations are associated with high mutation burden related to tobacco smoking. These lung cancers associated with smoking are genetically complex tumors as opposed to adenocarcinomas occurring in never smokers, which usually harbor an oncogenic driver targetable with a small-molecule tyrosine kinase inhibitor (TKI) such as EGFR and ALK TKIs.

Abbreviations: MET: hepatocyte growth factor receptor, NET: neuroendocrine tumor, NTRK: neurotrophic tropomyosin receptor kinase, RET: ret proto-oncogene, ROS1: ROS proto-oncogene 1, STK11: serine/threonine kinase 11 (also called LKB1).

1.4 Lung cancer treatment and prognosis

The treatment of lung cancer depends on the disease stage at diagnosis, the histology and the presence of a targetable oncogenic driver. The stage of the cancer refers to the extension of the cancer in the body.

1.4.1 Non-small-cell lung cancer

Localized disease (stage I-IIA according to the 8th TNM edition) (**Fig. 8**) refers to tumors involving only the lung and eventually ipsilateral hilar lymph nodes. It accounts for 26% of lung cancer cases [25] and the 5-year-overall survival is around 56-90% [26,27]. It is usually treated with local therapy, preferentially surgical resection. Stereotactic radiotherapy is an alternative if there are functional contraindications to surgery and if the tumor is small, without extension to hilar lymph nodes.

Locally-advanced disease (stage IIB-III according to the 8th TNM edition) (**Fig. 8**) refers to a lung tumor invading mediastinal lymph nodes. It accounts for 33% of lung cancer cases [25] and the 5-year overall survival is 37% [26-28]. It is usually treated with multimodal therapies, for instance surgery resection after neoadjuvant chemotherapy or thoracic radiotherapy combined with chemotherapy followed by immunotherapy consolidation (**Fig. 8**).

Metastatic disease (stage IV according to the 8th TNM edition) (**Fig. 8**) refers to a tumor with invasion of extrathoracic organs or, in the thorax, to pleura or pericardium. It accounts for 49% of lung cancer cases [26,27] and the 5-year overall survival is 15% [25,29]. Systemic treatment is the cornerstone treatment of the metastatic disease [30,31]. It includes platinum-based doublet chemotherapy, immunotherapy with immune checkpoint inhibitors (ICIs), and tyrosine kinase inhibitors (TKIs) targeting oncogenic abnormalities (**Fig. 8**).

The systemic treatment of advanced-stage NSCLC has experienced many breakthroughs since 2004. A better understanding of NSCLC biology and especially genomics led to the identification of targetable oncogenic drivers such as EGFR, HER2, BRAF V600, and MET exon 14 skipping mutations, or ALK, ROS-1, RET, and NTRK rearrangements (Fig. 7). NSCLCs harbouring these targetable oncogenic drivers represent one in five patients in Western countries [23]. These oncogenic alterations occur mainly in non-squamous NSCLCs and never-smokers [23]. They confer sensitivity to targeted therapies which are TKIs. TKIs have brought remarkable improvements in response rates, progression-free survival, and intracranial disease control in patients with advanced-stage NSCLC harbouring oncogenic abnormalities. For instance, the median PFS with historical chemotherapy is 5.4–6.9 months in the first-line setting, compared to 18.9 months with osimertinib (a third-generation EGFR TKI) and 25.7 months with alectinib (a second-generation ALK TKI). Moreover, TKIs have a favourable toxicity profile compared to standard chemotherapy. Currently, there are four genetic alterations for which we have FDA-approved targeted therapies with TKIs: EGFR mutations, ALK rearrangements, ROS1 rearrangements, and BRAF V600 mutations which account for 15%, 5%, 2% and 2% of NSCLC, respectively [23,32-34]. Of note, EGFR mutations are much more

frequent in Asian patients suffering from NSCLC, found in approximately 50% of the cases [33,35].

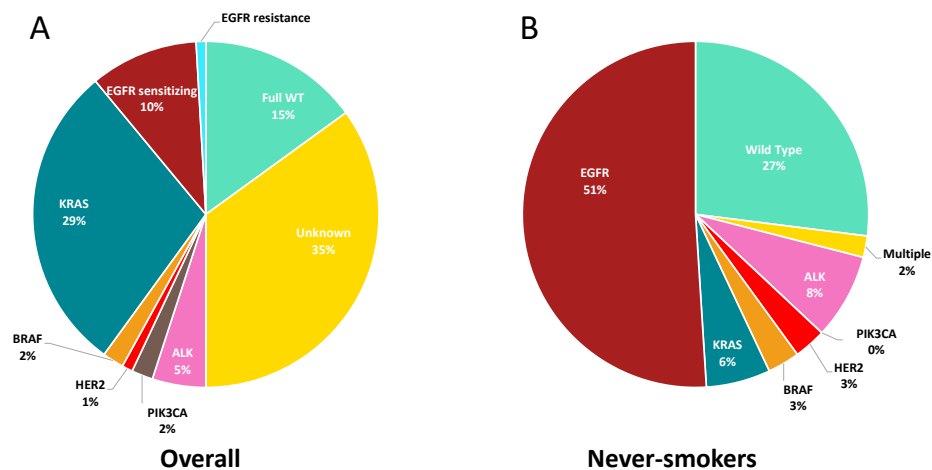


Figure 7: Frequency of molecular alterations in six genes in A: 18,679 NSCLC samples and B: 384 NSCLC samples from never smokers. Data are presented as percentages.

Abbreviations: ALK: anaplastic lymphoma kinase; BRAF: v-Raf murine sarcoma viral oncogene homologue; EGFR: epidermal growth factor receptor; KRAS: Kirsten rat sarcoma viral oncogene homologue; HER2 (also known as ERBB2): human epidermal growth factor receptor 2; IFCT: French Cooperative Thoracic Intergroup PIK3CA: phosphatidylinositol-3-kinase, catalytic subunit α . Data from IFCT [23,24].

Over the past decade, research in cancer immunology also led to a second breakthrough, namely the discovery of abnormalities in immune checkpoints, responsible for the immune evasion of cancer cells. This led to the development of ICIs that changed the paradigm of NSCLC management. Immunotherapies targeting Programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) release T lymphocyte-mediated immune responses by preventing the interaction between T cell inhibitory receptors and their ligands on tumor or tumor microenvironment cells [36,37]. Single agent ICIs have demonstrated a clear improvement in response rates and overall survival in unselected pretreated advanced-stage NSCLCs [31,38-41], as well as in untreated metastatic NSCLC with PD-L1 expression in $\geq 50\%$ cancer cells [31,42] and in consolidation of locally-advanced NSCLC after concurrent radiochemotherapy [28]. In patients with previously untreated metastatic NSCLC,

addition of an ICI to standard chemotherapy with or without antiangiogenic significantly increased overall survival compared to standard chemotherapy alone, independently of PD-L1 expression [43,44].

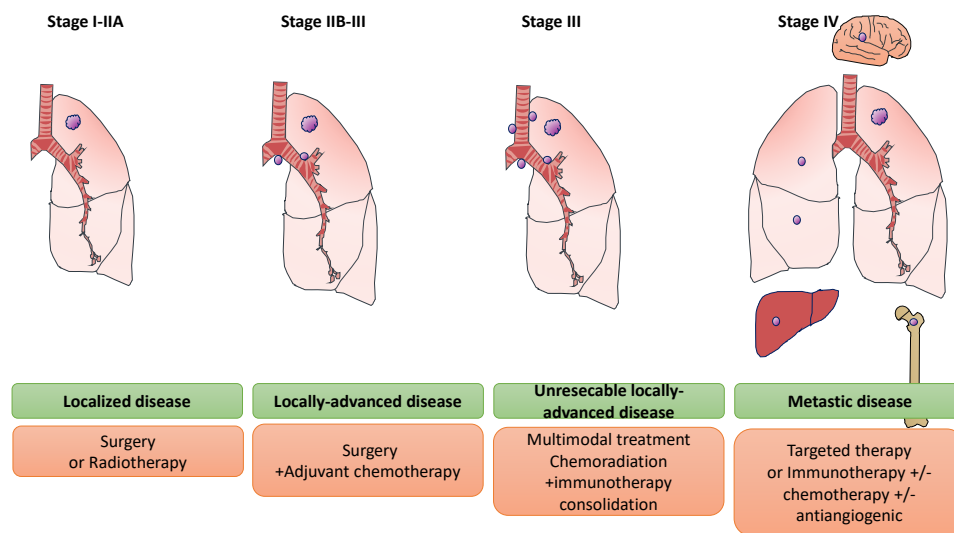


Figure 8: Management strategies of non-small-cell lung cancer according to disease stage.

1.4.2 Small-cell lung cancer

SCLC is broadly divided into limited-stage disease (LD) and extensive-stage disease (ED), which account for 14% and 86% of the cases, respectively [20,45]. LD refers to a tumor confined to one hemithorax and hilar/mediastinal lymph nodes. Combination of chemotherapy with thoracic radiation is the standard of care for LD, providing a median overall survival of 15-20 months. There is a place for surgery followed by adjuvant chemotherapy for very small (< 3 cm) LD-SCLC without lymph nodes involvement (**Fig. 9**), which accounts for only 6% of LD [46-53]. Objective response rates of 80-90% are achieved in LD-SCLC treated by concurrent radiochemotherapy [49,50]. Despite an impressive initial response to treatment, early recurrence is observed in almost all patients, explaining the poor survival.

1.4.2.1 Treatment options in first-line setting of small-cell lung cancer

ED refers to tumor spreading to contralateral lung, pleura, pericardium, or any extrathoracic organ (**Fig. 9**). The median overall survival of ED-SCLC treated with standard platinum-based chemotherapy is 8-10 months and less than 2% of ED-

SCLC patients are alive five years after diagnosis. There is a vigorous initial response of SCLC to frontline chemotherapy, with 60%-70% objective response rates, but this is also followed by early relapse leading to very poor outcomes [21,54]. The treatment of ED-SCLC disease has not experienced major breakthroughs over the past four decades. The standard first-line treatment of ED-SCLC remained platinum and etoposide chemotherapy from 1985 to 2019 [48,55-57]. Several trials with targeted therapies including Rova-T or immunotherapy (anti-PD-1 and anti-CTLA4 as monotherapy or in combination with chemotherapy) failed to significantly improve outcomes in ED-SCLC [58,59] despite antitumoral activity experienced in early phase trials [60,61]. In 2019, after decades of negative trials, the IMpower133 trial comparing carboplatin plus etoposide with or without atezolizumab (PD-L1 inhibitor) in first-line treatment of patients with ED-SCLC showed an improved overall survival in patients receiving atezolizumab in addition to chemotherapy compared to those receiving chemotherapy only. Based on this study, atezolizumab combined with platinum and etoposide chemotherapy has become the new standard of care for the first-line treatment of ED-SCLC since 2019 (**Fig. 9**). However, even though positive, these results are disappointing because there is only a two-month improvement in median overall survival [62].

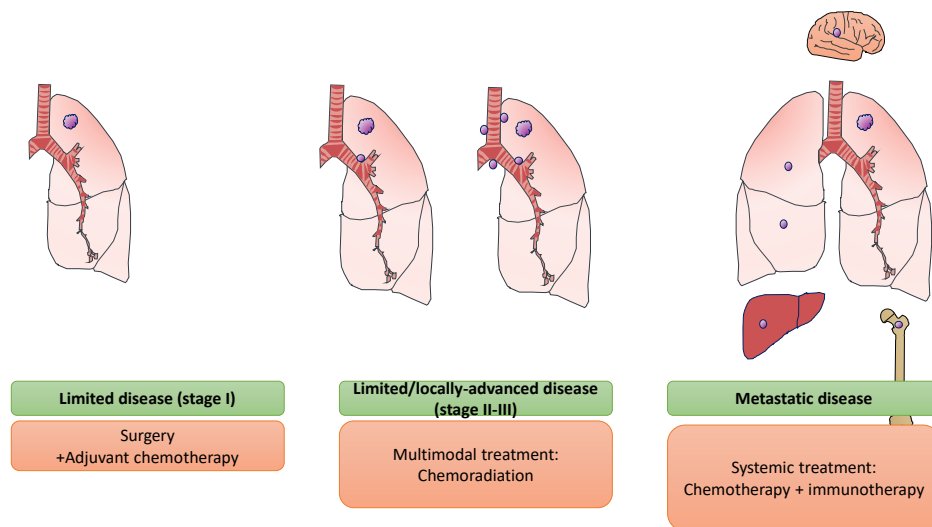


Figure 9: Management of small-cell lung cancer according to disease-stage.

1.4.2.2 Second-line and subsequent therapy of small-cell lung cancer

SCLC is very sensitive to first-line platinum and etoposide chemotherapy. However, despite this efficacy, the majority of ED patients relapse within 5-6 months with a

disease that often became resistant to first-line therapy, leading to a median survival of 12–13 months [62,63].

Second-line and subsequent therapies are considered at the time of progression of disease because it provides significant palliation in some patients [48,64]. Patients with sensitive relapse may derive benefit from reintroduction of the first-line regimen (usually platinum–etoposide) [48,65,66]. Compared to best supportive care, oral topotecan leads to improvement in median overall survival (OS)(25.9 versus 13.9 weeks), a slower quality of life decline, and greater symptom control [67]. Among patients with sensitive disease following completion of first-line therapy, oral and IV topotecan demonstrated similar efficacy and tolerability [68]. Oral or IV topotecan (**Fig.10**) is recommended for patients with resistant or sensitive relapse, while cyclophosphamide, doxorubicin or doxorubicin and vincristine (CAV) (**Fig.10**) is an alternative option [48]. Refractory or sensitive disease are defined as progression or recurrence occurring ≤ 3 or > 3 months, respectively, after completion of first-line therapy. It has been shown that the overall response rates (ORR) in the refractory and sensitive groups treated with topotecan in second-line setting were 6.4% and 37.8%, respectively. The median OS in the groups was 4.7 and 6.9 months [69]. Furthermore, a randomized study compared IV topotecan to CAV [70]. ORRs in the topotecan and CAV arms were 24.3% and 18.3%, respectively, though not statistically different [70].

Multiple other chemotherapies have shown activity comparable to topotecan in the second-line setting, including irinotecan [71], paclitaxel [72,73], docetaxel [74], gemcitabine [75,76], vinorelbine [77,78], temozolomide [79,80], oral etoposide [81,82], Lurbinectedin [83], and Bendamustine [84] (**Fig.10**).

Nivolumab as monotherapy and in combination with ipilimumab (**Fig.10**) provided durable responses (median duration of response: 4.4 months vs 7.7 months) and encouraging survival [60,85] and are now among the NCCN-recommended regimens for relapsed SCLC not previously treated with anti-PD-1 /PD-L1 therapy [86]. Patients who require third-line or subsequent therapy for advanced SCLC have limited treatment options. In these patients, pembrolizumab has demonstrated durable antitumor activity if not previously treated with anti-PD-1 /PD-L1 therapy [87].

Moreover, unlike NSCLC, there is currently no validated targeted therapy against an oncogenic driver in SCLC, which is the consequence of a poor understanding of its biology. Angiogenesis is the pathway that has been mostly investigated in SCLC, with evaluation of several targeted agents. This highlights the need for intensive research in the field.

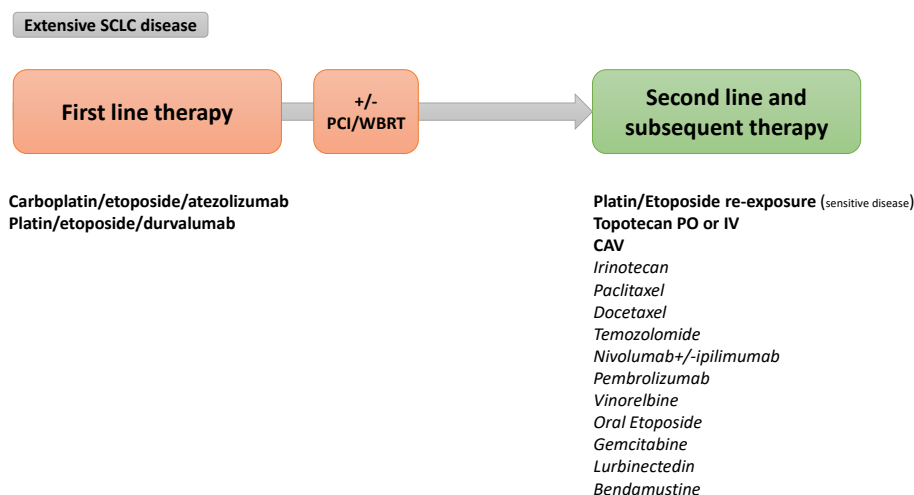


Figure 10: Treatment options in extensive-stage SCLC. Standard treatment in bold.

Abbreviations: CAV: cyclophosphamide, doxorubicin or doxorubicin and vincristine; PCI: Prophylactic cranial irradiation; WBRT: Whole brain radiotherapy.

1.4.2.3 Targeting angiogenesis in Small-cell lung cancer

The formation of new blood vessels, also known as angiogenesis, is crucial for tumour progression and metastasis [88]. Tumor growth and metastasis depend on angiogenesis and lymphangiogenesis which are triggered by angiogenic factors such as vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang1), basic fibroblast growth factors (bFGF), transforming growth factor- β 1 (TGF- β 1), and platelet-derived growth factor (PDGF) from tumor cells [89,90]. Targeting angiogenesis reduces the oxygen and nutrients supply necessary to facilitate tumor growth, as well as provide a route by which tumor cells can metastasize. Therefore, evaluate anti-angiogenic agents is particularly relevant in SCLC which is a tumor with high proliferation rate and high incidence of metastasis and circulating tumor cells [20,25,91-93]. Furthermore, it has been shown that most of SCLC (80%) display high microvessel density and VEGF expression [94,95]. High serum concentrations of VEGF and tumor VEGF overexpression have both been associated with poor response to chemotherapy and poor survival [96-100].

High levels of serum Ang-2, a member of the angiopoietin family which can promote vascular remodeling in concert with VEGF, have been reported and associated with poor survival in SCLC [101,102].

Anti-angiogenics agents include anti-VEGF agents such as monoclonal anti-VEGF antibody (bevacizumab), VEGF Trap (aflibercept), and small-molecule tyrosine kinase inhibitors (sorafenib, sunitinib, vandetanib, pazopanib, axitinib, and regorafenib, etc). Bevacizumab is a humanized monoclonal antibody that acts by binding and neutralizing all VEGF-A isoforms in the circulation. Aflibercept is a recombinant fusion protein with a high affinity binding domain from the extracellular domain of VEGFR1 and VEGFR2 fused to the Fc fragment of human immunoglobulin G1. It binds to VEGF-A, VEGF-B and placental growth factor (PIGF), subsequently preventing ligand binding to VEGFR-1 and VEGFR-2 [103]. In contrast to bevacizumab, small-molecule TKIs prevent activation of VEGFRs, thus inhibiting downstream signaling pathways rather than binding to VEGF directly. Many of these agents also have additional TKI activity against receptors such as epidermal growth factor receptor (EGFR) or c-kit, which may produce a direct antitumor effect. Vandetanib is an orally available inhibitor of two key pathways in tumor growth: VEGFR-dependent tumor angiogenesis and EGFR-dependent tumor cell proliferation and survival [104]. Sunitinib is an oral tyrosine kinase inhibitor, including VEGFR, KIT, and PDGF receptor (PDGFR) [105]. Cediranib (AZD-2171) is a highly potent and orally active inhibitor of VEGFR-2 tyrosine kinase activity, and demonstrates excellent selectivity versus a range of additional kinases. AZD-2171 inhibits VEGF-induced VEGFR-2 phosphorylation [106]. Sorafenib is a potent inhibitor of Raf-, and also active against VEGFR-2, VEGFR-3, and PDGFR- β [107]. Nintedanib is a potent, orally available, angiokinase inhibitor with proven preclinical antiangiogenic and antitumor activity, that targets all subtypes of VEGF receptor, PDGFR and FGF receptor (FGFR) [7], as well as ret proto-oncogene (RET) and Fms-like tyrosine kinase3 (FLT3) [108]. Thalidomide is an inhibitor of angiogenesis induced by bFGF and suppress tumor necrosis factor α (TNF- α) production from macrophages [109]. Pazopanib is an oral angiogenesis inhibitor targeting VEGFR-1/2/3 and PDGFR- α/β and c-Kit [110].

Several anti-angiogenics have been investigated for the treatment of ED SCLC, but these have shown only modest effects on survival and limited efficacy in different settings as recapitulated in **Table 1**.

Agent	Reference	Phase	N	Trial design	ORR (%)	PFS (months)	OS (months)
Bevacizumab	Ready et al. [111]	II	64	CDDP/CPT-11/Bev	75	7	11.6
	Spigel et al. [112]	II	102	EP ± Bev	58 vs. 48 (p=0.3269)	5.5 vs. 4.4 (HR=0.53)	9.4 vs. 10.9 (HR=1.16)
	Horn et al. [113]	II	65	EP/Bev	63.5	9.1 (TTP)	10.9
	Spigel et al. [114]	II	51	Carbo/CPT-11/Bev	84	5.5 vs. 5.3 (p=0.082)	12.1
	Pujol et al. [115]	II/III	147	EP or PCDE ± Bev	89.2 vs. 91.2, (p=1.00)	7.8	13.3 vs. 11.1 (p=0.35)
	Petrioli et al. [116]	II	22	EP/Bev	77.2	3.38	13.2
	Jalal et al. [117]	II	34	Paclitaxel/Bev (pretreated)	18.1	2.7	6.9
	Mountzios et al. [118]	II	-	Paclitaxel/Bev (pretreated)	20	6.24 (sensitive)	6.3
	Spigel et al. [119]	II	50	Topotecan/Bev (pretreated)	16	2.91 (refractory)	7.4
Sunitinib	Schneider et al. [120]	II	60	EP → sunitinib maintenance	0	2.5, (start of sunitinib)	8.4 (start of treatment)
	Spigel et al. [121]	II	34	Carbo/CPT-11 → sunitinib maintenance	59 (overall)	7.6 (TTP)	1-year OS 54%
	Ready et al. [122]	II	138	EP → sunitinib vs. placebo	-	3.7 vs. 2.1 (p=0.02)	9 vs. 6.9 (p=0.16)
	Han et al. [123]	II	25	Second-line, monotherapy	9	1.4	5.6
Rh-endostatin	Lu et al. [124]	II	140	CE ± rh-endostatin	86.7 vs 71,0 (p=0.046)	6.4 vs 5.87 (p=0.21)	12.1 vs 12.4 (p=0.81)
Thalidomide	Pujol et al. [125]	III	119	PCDE ± thalidomide	87 vs. 84,0 (p=0.69)	6.6 vs. 6.4 (p=0.15)	11.7 vs. 8.7 (p=0.16)
	Lee et al. [126]	III	724	CE ± thalidomide	74 vs. 72 (NS)	7.6 vs. 7.6 (p=0.39)	10.1 vs. 10.5 (p=0.28)
Aflibercept	Allen et al. [127]	II	192	Topotecan ± aflibercept (pretreated)	2 vs. 0 (p=0.50)	1.6 vs. 1.3 (p=0.02)	5.4 vs. 4.4 (p=0.34)
				Chemotherapy vandetanib vs. placebo → maintenance	NR	2.7 vs. 2.8 (p=0.51)	10.6 vs. 11.9 (p=0.9)
Cediranib	Ramalingam et al. [129]	II	25	Second-line	0	2	4
Sorafenib	Gitlitz et al. [130]	II	83	Second-line	6	2.2 (sensitive)	6.7 (sensitive)
						2.0 (refractory)	5.3 (refractory)
Pazopanib	Koinis et al. [131]	II	58	Second-line	18 (sensitive)	5.5 (sensitive)	8.0 (sensitive)
					5.2 (refractory)	2.0 (refractory)	4.0 (refractory)
Nintedanib	Han et al. [132]	II	22	Second-line, third-line	5	1.0	9.8

Table 1: Clinical trials evaluating anti-angiogenic treatments in SCLC

Abbreviations: Bev, bevacizumab; CDDP, cisplatin; CE: carboplatin/etoposide; CPT-11, irinotecan; DCR, disease control rate; EP, etoposide/cisplatin; PCDE, cisplatin, cyclophosphamide, epidoxorubicin, etoposide; HR, hazard ratio; sensitive; NR, not reported; NS, statistically non-significant; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; refractory, platinum refractory relapse; rh-Endostatin, recombinant human endostatin ; SCLC, small cell lung cancer; TTP, time to progression; sensitive: platinum sensitive relapse. Adapted from [133].

1.5 Towards a new molecular classification of small-cell lung cancer

In the past decade, the therapeutic management of NSCLC has shifted from a histology-based approach towards a molecularly-driven approach. First, targeted therapies with TKIs in patients with NSCLCs harbouring an oncogenic abnormality significantly improved their outcome, with moreover a favourable toxicity profile as compared with chemotherapy. Then, immunotherapy with PD-L1 ICIs improved overall survival both in unselected pre-treated patients and in untreated patients with advanced NSCLCs, with better results however in presence of PD-L1 expression in $\geq 50\%$ cancer cells. As opposed to NSCLC, progress in the understanding of SCLC biology has been slower and more limited. This translated into the absence of efficient targeted therapies against oncogenic drivers and more disappointing results with ICIs in SCLC. Furthermore, in comparison to chest radiography screening of individuals at high risk for developing lung cancer, early detection using low-dose CT (LDCT) in the National Lung Screening Trial (NLST) reduced mortality from lung cancer as well as overall mortality by 20% and 6.9%, respectively [134]. Lung cancer mortality reduction with LDCT screening has also been observed in the NELSON and MILD trials [135-137]. While lung cancer screening with LDCT had impact on NSCLC outcome, this benefit was not found for SCLC because of the high proportion of interval detected cancers (diagnosed within one year of a negative screen) and advanced-stage disease (III/IV) detected among SCLC (86%) as compared to NSCLC (36%) [91,134]. The poor understanding of SCLC biology and the lack of early detection methods has translated into the absence of breakthroughs in the care and targeted therapy in SCLC patients over the last 30 years.

SCLC is classified in the family of pulmonary neuroendocrine tumours (PNETs), which includes a spectrum of tumors with significant clinical, histological, biological, and molecular differences. PNETs account for 20-25% of all lung cancers and encompass well-differentiated and poorly differentiated tumors [138-140]. Well-differentiated PNETs, also known as pulmonary carcinoids, include typical (TC) and atypical (AT) carcinoids. Poorly differentiated aggressive high-grade neuroendocrine carcinomas (HGNECs) are subdivided into large-cell neuroendocrine carcinomas (LCNECs) and SCLC [141,142]. LCNEC and SCLC are associated with heavy smoking history [143] in contrast to TCs which usually occur in young never or light current smokers (**Fig. 5**). ATs usually develop in former or current smokers [144-146].

SCLC accounts for 15% of all lung cancers. It is a highly aggressive disease characterized by a high growth rate, a fast doubling time, and a tendency for early metastasis. For instance, patients with ED-SCLC represent 86% of all the cases at the time of diagnosis. The median survival of ED-SCLC is 8 to 10 months and the 5-

year overall survival is as low as 2% [55]. The 5-year overall survival of LD-SCLC treated with concurrent chemoradiotherapy is 35% [147]. Only modest improvements have been made in survival over the last decades [148].

SCLC is predominately localized (**Fig. 9**) in the central airways and mediastinal lymph nodes spreading is often observed at diagnosis. Tumor cells typically express neuroendocrine markers such as chromogranin, synaptophysin, gastrin-releasing peptide, and CD56. For this reason, SCLC cells have been thought to derive from neuroendocrine cells. It has been well established that neuroendocrine cells which predominately localize to bronchioles are the predominant cells of origin of SCLC [149]. But SCLC can also derive from the alveolar type 2 epithelial cells, displaying in this case a peripheral location (**Fig. 5**) [149].

In contrast to NSCLC [23,150], the frequency of potentially actionable genomic alterations in SCLC is lower, with an average of 0.98 targetable genomic alterations per patient. Approximately 25% of the altered genes in SCLC are currently considered to be actionable [151]. Most of the mutations observed in solid tumors as well as in SCLC tumors have no effect on the neoplastic process and are known as passenger mutations [152]. Tobacco exposure in SCLC leads to a high number of genomic alterations, including single-nucleotide substitutions, small insertions of one or a few nucleotides (indel) that may substitute, produce a stop codon, or alter the encoded amino acid sequence of a protein. Other genetic alterations producing many copies of a small segment of the genome (amplification) or generating rearrangements where two regions or genes from two nonhomologous chromosomes are joined (inter and intrachromosomal rearrangements or translocations) can also be found in SCLC as in other solid tumors. All these genetic alterations can inactivate or activate specific genes (tumor suppressor genes (TSGs) or oncogenes, respectively) and therefore increase the selective growth advantage of the cells in which they occur [7,152]. About 95% of these mutations are single-base substitutions, whereas the remaining 5% are deletions or insertions of one or a few bases, translocations, and amplifications (**Fig.11**) [148,152,153]. Signaling pathways recurrently affected in SCLC are broadly divided into four pathways involved in cell cycle regulation, transcriptional regulation, receptor kinase PI3K and Nocth signalings, and neuroendocrine differentiation [148].The most common genomic alterations encountered in SCLC occur in TSGs, including tumor protein 53 (TP53) and retinoblastoma associated protein 1 (RB1), inactivating the TSGs and therefore increasing the selective growth advantage of the cell [152]. Mutual inactivation of both alleles of TP53 and RB1 affects cell cycle regulation pathways and seems to be a crucial molecular event that drives SCLC pathogenesis (**Fig.11 and 12**). Mutual bi-allelic loss of TP53 and RB1 is found with novel generation sequencing approaches in approximately 100% and 93% of SCLC cases, respectively (**Fig.11 and 12**) [148,151,153,154]. Other TSG inactivating alterations,

including mixed-lineage leukemia (MLL2) (17%), phosphatase and tensin homolog (PTEN) (6-9%), cyclin-dependent kinase inhibitor 2A (CDKN2A) (5%), and fragile histidine triad diadenosine triphosphatase (FHIT) (located in chromosome 3p), are less frequent in SCLC (**Fig.11 and 13**) [148,151,153,155]. Somatic alterations including the spectrum of TP53 mutations with C:G>A:T transversions are linked to polycyclic aromatic hydrocarbons and acrolein in tobacco smoke, which are uncommonly observed in lung cancers from non-smokers [7,148,154,156,157]. The complete genomic inactivation of both TP53 and RB1 function is required in the pathogenesis of SCLC (**Fig.11 and 12**). This has been proven in mouse models where mutual loss of TP53 and RB1 efficiently transformed neuroendocrine or alveolar type 2 cells into SCLC. It has also been shown that neuroendocrine cells are the predominant cells of origin of SCLC in comparison to alveolar type 2 cells [149,158].

Unlike oncogenes, genomic alterations that occur in TSGs generate proteins with inactivated function, which are non-targetable so far. In addition, TSG inactivating alterations predominate over oncogene activating mutations in the most common solid tumors, including SCLC [148,151-153]. While approximately 60% of Asian patients and 21-26% of Caucasian patients suffering from NSCLC harbour currently targetable oncogene alterations, this is not the case in SCLC [150]. In contrast to NSCLC, tyrosine kinase pathway genes, including KRAS, EGFR, and ALK (**Fig. 7**) are rarely altered in SCLC. Most common oncogenic alterations (base substitutions, amplifications, or homozygous deletions) encountered in SCLC include amplification of avian myelocytomatosis viral oncogene homolog (MYC) family members (20%), rearrangements of tumor protein 73 (TP73) (13%), amplification of the tyrosine kinase genes fibroblast growth factor receptor 1 (FGFR1) (6%), KIT (also known as CD117) (6%), and of insulin receptor substrate 2 (IRS2) (2%), and mutations of phosphatidylinositol-3-kinase catalytic subunit α (PIK3CA) (3%) (**Fig.13**) [148]. Amplification in sex determining region Y box 2 (SOX2) transcription factor has also been reported in SCLC [151,154]. Nevertheless, none of these oncogenic drivers have been successfully targeted in SCLC so far.

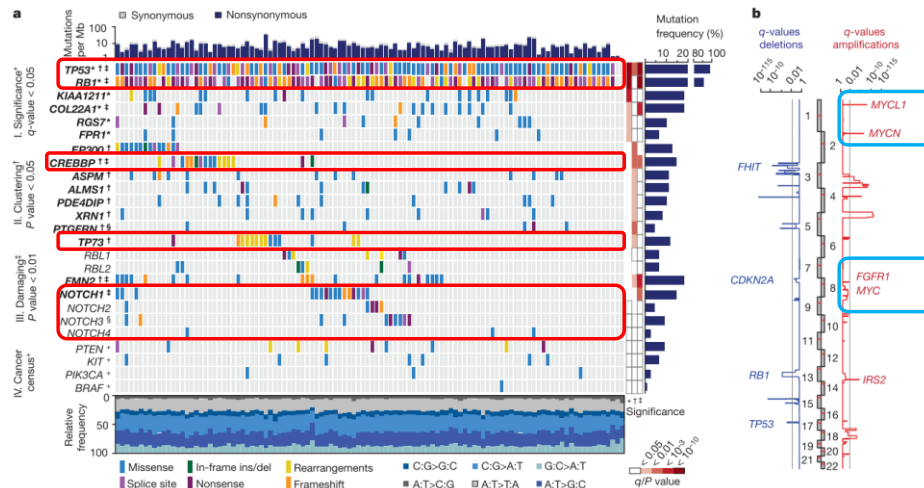


Figure 11: Genomic alterations in small-cell lung cancer. Adapted from [148].

a. Somatic mutations in SCLC. Nearly universal and biallelic loss of TP53 and Rb1. Recurrent mutations or translocations inactivation mutations in CREBBP (15% of SCLC) as well as TP73 gene (13% of SCLC) are common alterations in SCLC. Inactivation mutations in NOTCH genes are found in 25% of SCLC. Mutations in CREBBP, TP73, and NOTCH family genes are mutually exclusive.

b. Somatic copy number alterations determined for 142 human SCLC tumours by single nucleotide polymorphism (SNP) arrays. Significant amplifications (red) (MYC family genes, FGFR1, and IRS2) and deletions (blue) (FHIT, CDKN2A, TP53 and RB1) were determined for the chromosomal regions and are plotted as q-values significance (0.05).

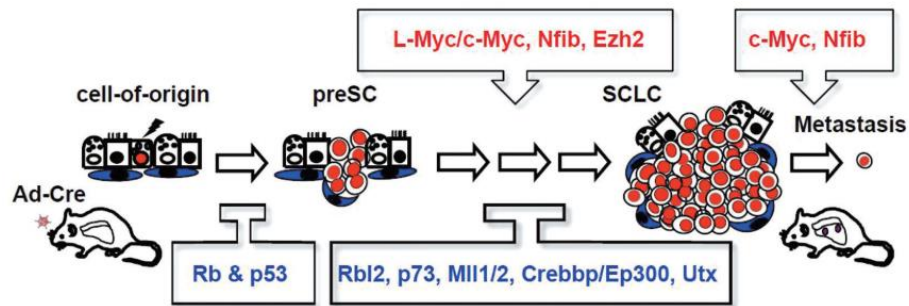


Figure 12: Major genetic events and their roles in SCLC development and progression (from [159]). A model of SCLC development in the Rb/p53 conditional-mutant mice. Pulmonary neuroendocrine cells are likely cells-of-origin. Validated or potential tumor suppressors and oncogenic drivers are indicated in blue and red, respectively.

Abbreviations: Crebbp: cAMP response element-binding (Creb)binding protein; Ezh2: Enhancer of zeste homolog 2; Mll: mixed lineage leukemia; Nfib: nuclear factor I b family of transcription factors; Utx: Ubiquitously transcribed tetratricopeptide repeat, X chromosome.

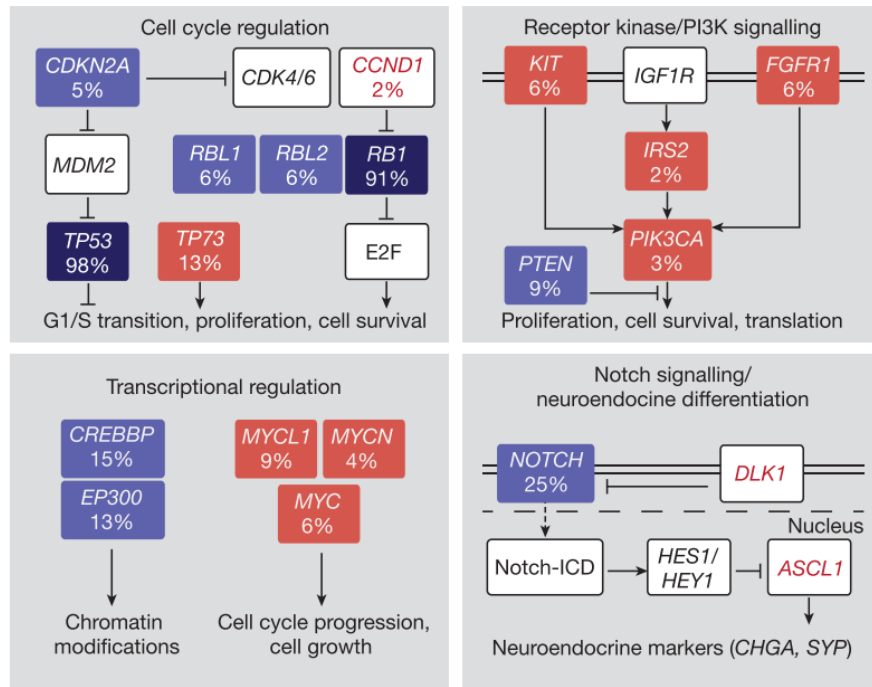


Figure 13: Signaling pathways recurrently affected in SCLC. Red and blue boxes denote genes with activating and inactivating alterations, respectively. Deep blue boxes highlight the bi-allelic inactivation of TP53 and RB1. Genes found expressed at high levels are shown in red font. Adapted from [148].

In summary, molecular alterations found in SCLC recurrently affect four signaling pathways. The cell cycle regulation pathway which involves bi-allelic inactivation of TP53 and RB1 is present in almost all SCLC. In this pathway, other genetic alterations could inactivate TP73, RBL1 or RBL2 genes as well as CDKN2A deletion which results in protein loss function is also found in 5 % of SCLC. These molecular alterations related to cell cycle regulation pathway confer proliferation and cell survival advantages to SCLC (**Fig.13**).

The Receptor kinase/PI3K signaling activation is triggered by PTEN inactivation (9 % of SCLC) or FGFR1 (6 % of SCLC), KIT (6 % of SCLC), IRS2 (2 % of SCLC) gene amplification or PIK3CA (3% of SCLC) activating mutation (**Fig.13**). Transcriptional regulation pathway in SCLC mainly involves CREBBP inactivation and MYC family gene amplification which promote chromatin modification and promote cell cycle

progression and cell growth, respectively (**Fig.13**). Inactivating mutations occurring in NOTCH family genes are found in 25% of SCLC and point out the role of Notch signaling and the neuroendocrine differentiation pathway in this disease (**Fig.13**).

Recently, some breakthroughs in the molecular and cellular biology of SCLC have been achieved with the identification of three subsets of SCLC driven by two transcription factors, achaete-scute homolog 1 (ASCL1; also known as ASH1) and neurogenic differentiation factor 1 (NEUROD1) that are required for cell survival in disease. The classic and most frequent subset of SCLC, accounting for 70% of the cases, is characterized by high expression of ASCL1. The variant subset, representing 11% of the cases, expresses high levels of NEUROD1 and low levels of neuroendocrine markers [160,161]. The third subset of SCLC, which represents only 19% of the cases, does not express ASCL1 or NEUROD1 and therefore lacks classic and variant SCLC neuroendocrine markers [162,163]. This third subtype, also known as the non-neuroendocrine subtype, has recently been linked to two transcriptional drivers, the POU class 2 homeobox 3 (POU2F3), and yes-associated protein 1 (YAP1), which account for 16% and 2% of all SCLC, respectively [164-166] (**Fig. 14**).

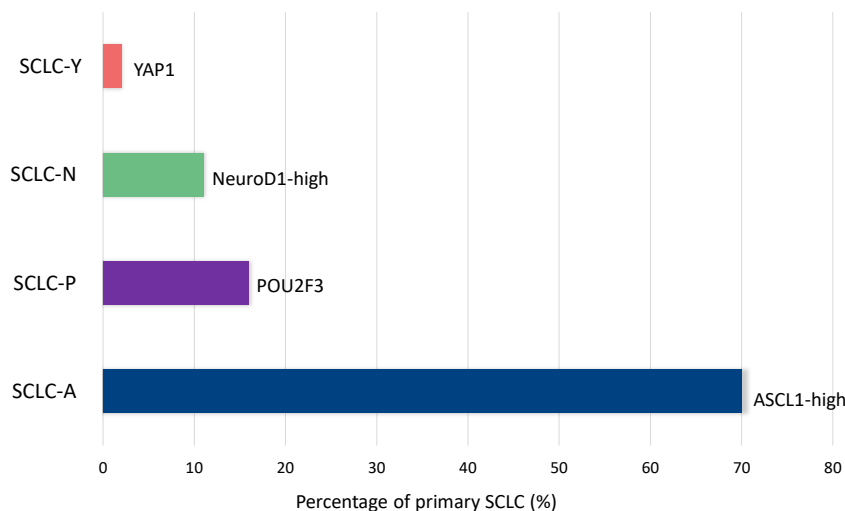


Figure 14: Proportion of small-cell lung cancer molecular subtypes, defined based on the expression of the following key transcription regulators: achaete-scute homologue 1 (ASCL1) for the classic subtype; neurogenic differentiation factor 1 (NeuroD1) for the variant subtype; POU class 2 homeobox 3 (POU2F3) and yes-associated protein 1 (YAP1) for the non-neuroendocrine subtype.

ASCL1 and NEUROD1 are transcription factors that play an important role during neurogenesis [167-170]. They can also reprogram other somatic cell types into neurons [171,172]. They are essential for the development of normal lung neuroendocrine cells as well as other endocrine and neural tissues [167,168,173,174]. Overexpression of ASCL1 and NEUROD1 has been reported in several neuroendocrine cancers including aggressive neuroendocrine lung cancers [160,175-179]. It has been established that ASCL1 and NEUROD1 play crucial roles in promoting malignant behavior and survival of human SCLC, and preclinical studies have shown that ASCL1 and NEUROD1 are required for growth and survival of SCLC cells [179-181]. Mice model with simultaneous loss of TP53 and RB1 function develop classical SCLC that co-express high levels of both ASCL1 and neuroendocrine markers, such as calcitonin gene-related peptide (CGRP) and synaptophysin (**Fig.15**) [163]. ASCL1 is a transcriptional activator directly targeting known oncogenes for SCLC, including Notch signaling components such as the gene encoding the inhibitory Notch ligand Delta-like protein 3 (DLL3) [163]. In contrast to other Notch ligands, DLL3 inhibits Notch signaling [182,183]. Moreover, NOTCH induces rapid degradation of ASCL1 by polyubiquitination-mediated degradation (**Fig.15**) [184]. Genomic alterations that invalidate NOTCH family genes are found in 25% of human SCLCs [148]. Thus, NOTCH inactivation by genomic alteration as well as DLL3 overexpression lead to ASCL1 expression that drives the classic SCLC phenotype, which represents the majority of SCLCs. Moreover, high DLL3 protein expression has been reported in 85% of recurrent/refractory and 72% of untreated SCLC patients [185]. DLL3 expression on the surface of SCLC and LCNEC tumour cells [185] has become a target of interest as well as a potential predictive biomarker for SCLC. Rovalpituzumab tesirine (Rova-T) is an antibody-drug conjugate (pyrrolobenzodiazepine (PBD)dimer cytotoxic) directed against DLL3 that binds to DLL3 expressed on the tumor cell surface. The antibody-drug conjugate internalization results in tumour-specific DNA damage and cell death. Rova-T (**Fig. 15**) has demonstrated encouraging preclinical and clinical results, with a strong correlation between the level of DLL3 expression and the therapeutic activity in early-phase clinical trials [61,185]. Unfortunately, Rova-T underperformed in the phase 2 SCLC TRINITY trial which included pre-treated patients with high levels of DLL3 on tumor cell surface [186]. The results were also disappointing in the phase 3 TAHOE trial (NCT03061812) exploring Rova-T versus topotecan in patients with advanced or metastatic SCLC with high levels of DLL3 and who had first disease progression during or following frontline platinum-based chemotherapy [187]. Nevertheless, the ongoing phase 3 MERU trial (NCT03033511) is exploring Rova-T as maintenance therapy following first-line platinum-based chemotherapy in patients with advanced or metastatic SCLC with high levels of DLL3. There are several DLL3-targeted agents in development, including a bispecific antibody AMG 757 that links DLL3-

positive tumor cells to CD3-positive T cells, aiming to recruit T cells into the tumor specifically (NCT03319940).

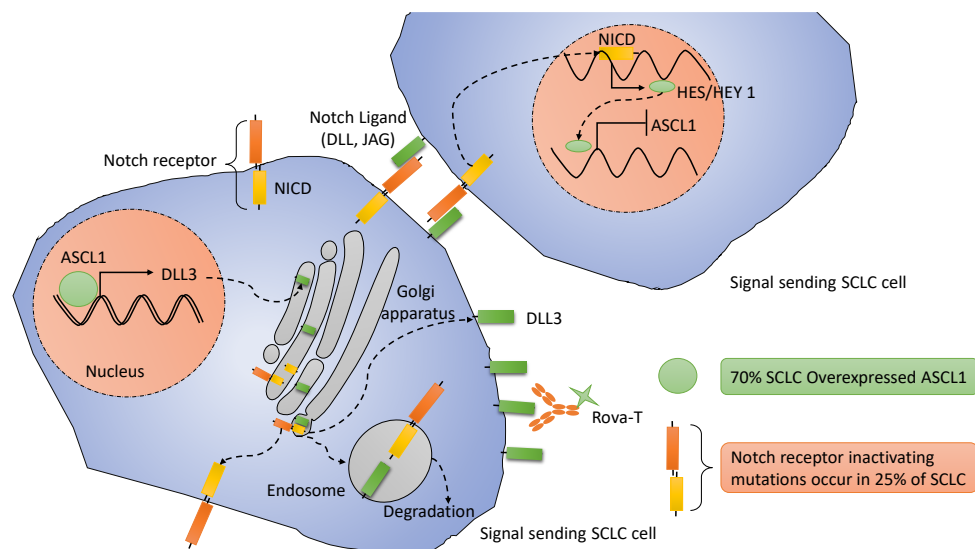


Figure 15: Achaete-scute homologue 1 (ASCL1) small-cell lung cancer subtype. Notch signaling is activated by the binding of a Notch receptor to the extracellular domain of a Notch ligand (DLL, JAG) expressed on the SCLC cell surface. Notch receptor binding with its ligand generates cleavage of the Notch receptor, resulting in release of the Notch intracellular domain (NICD) which promotes ASCL1 inhibition through HES/HEY 1. Most SCLCs harbor Notch inactivation or ASCL1 overexpression which leads to DLL3 overexpression on the cell surface in approximately 70% of SCLCs. Adapted from [181,183,185,188]

Abbreviations: ASCL1, achaete-scute homolog 1; DLL, delta-like ligand; JAG, jagged; NICD, Notch intracellular domain; HES 1, Hairy and enhancer of split 1; HEY 1, enhancer of split-related protein 1; ROVA-T, Rovalpituzumab tesirine a DLL3-targeted antibody-drug conjugate; SCLC, small-cell lung cancer.

The variant SCLC subset expressing NEUROD1 tends to have high c-MYC expression. It has been shown in mice that MYC drives a neuroendocrine-low variant subset of SCLC with high NEUROD1 expression. Furthermore, this SCLC with high MYC expression is targetable by Aurora kinase inhibition alone or combined with chemotherapy [189]. Importantly, 20% of SCLCs harboring alterations of the MYC

gene family members could thus potentially be actionable with Aurora kinase inhibitors [148,164].

1.6 Role of focal adhesion kinase in small-cell lung cancer and its potential as a therapeutic target

(modified from Aboubakar Nana, F et al. *Cancers* **2019**, 11, 1683)

Despite improved understanding of the molecular steps leading to SCLC development and progression these last years, there is still no effective targeted therapies in SCLC. After four decades, the only modest improvement in overall survival (OS) of patients suffering from ES-SCLC has recently been shown in a trial combining atezolizumab, an anti-PD-L1 immune checkpoint inhibitor, with carboplatin and etoposide, chemotherapy agents. This highlights the need to pursue research efforts in this field.

Focal adhesion kinase (FAK) is a non-receptor protein tyrosine kinase overexpressed and activated in several cancers, including SCLC, and contributing to cancer progression and metastasis through its important role in cell proliferation, survival, adhesion, spreading, migration, and invasion. FAK also plays a role in tumor immune evasion, epithelial-mesenchymal transition, DNA damage repair, radioresistance, and regulation of cancer stem cells. FAK is of particular interest in SCLC, known for its aggressiveness. Inhibition of FAK in SCLC cell lines demonstrated significant decrease in cell proliferation, invasion and migration, and induced cell cycle arrest and apoptosis. In this following review, we focus on the role of FAK in cancer cells and their microenvironment, and its potential as a therapeutic target in SCLC.

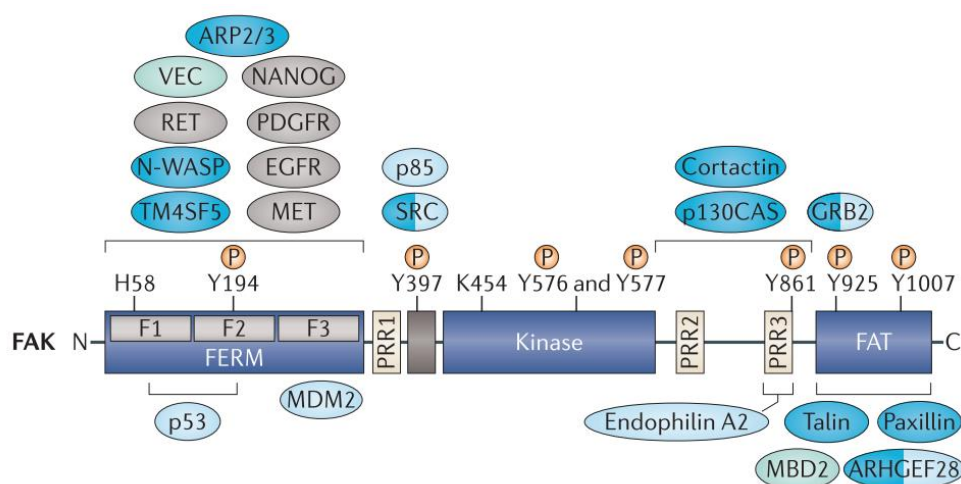


Figure 16: The focal adhesion kinase (FAK) domain structure. The protein band 4.1-ezrin-radixin-moesin (FERM) homology domain on the amino-terminal side. The kinase domain indicates the region of catalytic activity. PR1 and PR2 denote proline-rich regions 1 and 2 in the carboxylterminus. Important tyrosine phosphorylation (P) sites are indicated; Y397, K454 and H58 have crucial roles in FAK activation. FAT denotes the focal adhesion targeting domain. FRNK denotes the FAK-related non-kinase domain. Sites of tyrosine and serine phosphorylation are indicated. The amino-terminal PR domain is not shown. FAK binding partners are shown at their interaction sites within FAK. Binding of these proteins affects outcomes such as cell motility (dark blue), cell survival (light blue) or both functions (dark blue/light blue). Roles involving FAK activation are shown in grey, and important contributions to the tumour environment are shown in green. From [190]

Abbreviations: EGFR, epidermal growth factor receptor; GRB2, growth factor receptor-bound 2; MBD2, methyl-CpG-binding domain 2; N-WASP, neural Wiskott–Aldrich syndrome protein; PDGFR, platelet-derived growth factor receptor; TM4SF5, transmembrane 4 L6 family member 5; VEC, vascular endothelial cadherin .

1.6.1 Introduction

Focal adhesion kinase (FAK) is a 125 kDa non-receptor protein tyrosine kinase known to be overexpressed and activated in several cancers, including SCLC [191-199]. Unlike receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR), non-RTKs such as FAK are cytoplasmic enzymes that lack transmembrane and extracellular domains (**Fig.16 and 17**) [200]. FAK localizes to focal adhesions and is triggered on by extracellular signals such as integrin-mediated adhesion and G protein-coupled receptors (GPCR), growth factor receptors, and vascular endothelial growth factor receptor (VEGFR) [201]. Therefore, FAK plays a central role in the interaction between cells, including cancer cells, and their microenvironment. FAK structure includes an NH₂-terminal Protein4.1-ezrin-radixin-moesin (FERM) domain, a central kinase domain, two proline-rich motifs, and a COOH-terminal focal adhesion targeting (FAT) domain. FAK is maintained in an inactive state by the binding of the FERM domain to the kinase domain, which blocks access to the catalytic site and sequesters the activation loop, as well as the key autophosphorylation site tyrosine 397 (Tyr397) (**Fig.16 and 17**). Engagement of integrins with the extracellular matrix (ECM) or growth factors leads to signals that displace the FERM domain, resulting in rapid autophosphorylation of Tyr397, which is a critical event in signal transduction through FAK [201,202]. Tyr397 phosphorylation provides a binding site that recruits and activates Src through SH2 domains of Src family kinases. FAK-src complex

therefore maintains Src and FAK in their activated state, creating a functional kinase complex [203].

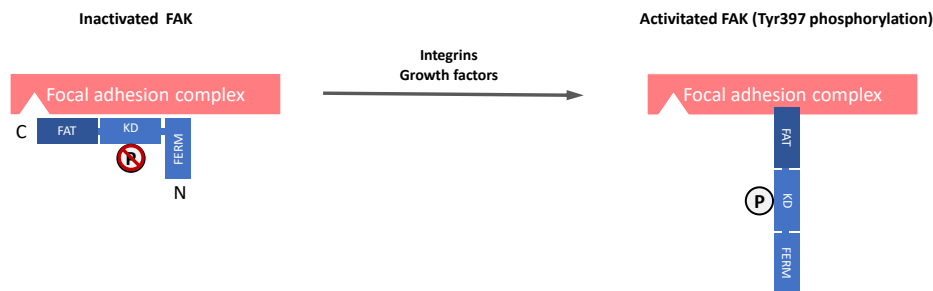


Figure 17: The domain organization and activation of focal adhesion kinase (FAK). FAK is composed of a central kinase domain (KD), an amino-terminal side flanked by a protein band 4.1-ezrin-radixin-moesin (FERM) homology domain, and a carboxy-terminal focal adhesion targeting (FAT) domain flanked by proline-rich regions (PRRs). FAK localizes to focal adhesions and is triggered off by extracellular signals such as integrin-mediated adhesion and some growth factors. FAK is maintained in an inactive state by the binding of the FERM domain to the kinase domain, which blocks access to the catalytic site and sequesters the activation loop, as well as the key autophosphorylation site tyrosine 397 (Tyr397). Engagement of integrins with the extracellular matrix (ECM) or growth factors leads to signals that displace the FERM domain, resulting in rapid autophosphorylation of Tyr397, which is a critical event in signal transduction by FAK.

Based on FAK overexpression and/or increased activity in cancer and its known function in multiple biological processes which play a role in the development and progression of cancers, such as crosstalk between cell and its microenvironment, potentially leading to cell growth, survival, adhesion, spreading, migration, invasion, angiogenesis, DNA damage repair, radioresistance, and regulation of cancer stem cells, it has been suggested that increased expression and/or activity of FAK may have a critical role in cancer development and progression [204]. FAK is therefore a potential target for anti-cancer therapy, especially in SCLC, known to be a highly invasive cancer. Small-molecule inhibitors targeting FAK kinase domain and preventing FAK activation (Tyr397 autophosphorylation) have been developed and some already entered early phase trials in different cancers (Table 1). Phase I trials with GSK2256098 [205-207], VS-6062[208], defactinib (VS-6063)[209-211], or BI853520[212-214] have shown an acceptable safety profile and favorable

pharmacokinetics (**Table 2**). Most frequent treatment-related adverse events included digestive disorders (nausea, diarrhea, vomiting), headaches, reversible proteinuria, and unconjugated hyperbilirubinemia [205-213]. With GSK2256098, a better response (stable disease) was observed in 37% of glioblastoma (3 patients, median PFS 5.7 weeks) [207] and in 45% of advanced solid cancers (28 patients) [206]. With VS-6062, 34% of patients (31 patients) with advanced solid tumors exhibited stable disease at 6 weeks, including one case of SCLC for ≥ 6 cycles [208]. VS-6063 led to stabilization of advanced solid tumors in 43% of Caucasian patients (6 cases) after 6 weeks of treatment [209] and in 33% of Asian patients (3 cases) during more than 24 weeks (median PFS of 63 days) [211]. Recently, the combination of the FAK inhibitor GSK2256098 and the MEK inhibitor trametinib in recurrent advanced pancreatic ductal adenocarcinoma did not provide significant clinical benefit in a phase II trial (PFS of 1.6 month and OS of 3.6 months) [215]. In malignant pleural mesothelioma, defactinib in maintenance after first-line chemotherapy in a phase II trial did not provide benefit either (PFS of 4.1 months with defactinib vs 4.0 months with placebo, and OS of 12.7 months with defactinib vs 13.6 months with placebo) [216]. Preoperative administration of defactinib in the ongoing phase II clinical trial NCT02004028 appears promising, with therapeutic benefit (13% objective partial response, 67% stable disease, 17% tumor progression) and beneficial modification of the tumoral microenvironment in mesothelioma [217]. For instance, defactinib increases naïve CD4 (CD45RA+PD-1+CD69+) and CD8 T cells, reduces myeloid and Treg immuno-suppressive cells, reduces exhausted T cells (PD-1+CD69+), reduces peripheral MDSCs; and histological subtype change of mesothelioma (pleomorphic or biphasic to epithelioid). Several clinical trials with defactinib associated with immunotherapy (NCT02758587, NCT03727880, NCT02943317), RAK/MEK inhibitor (NCT03875820), or chemotherapy (NCT02546531) are ongoing, some of them open to SCLC inclusion (**Table 2**) [205-208,210-216,218-232]. Other small-molecules targeting the protein-protein interactions between FAK and other proteins such as VEGFR-3, called scaffolding inhibitors, have been developed and shown to induce antitumoral effects in preclinical studies. Further research is needed to find predictive biomarkers of response to FAK TKI alone or, probably more promising, in association with another drug (**see below, page 45**).

In this review, we will focus on the role of FAK in tumor development and progression, and its potential as a therapeutic target in SCLC.

Drug /compagny	Type	Specificity	Cancers targeted	Study phase	References
TAE-226 Novartis	TKI ATP-C	FAK, IGF-IR, c-Met, Pyk2	Glioma, ovarian	Preclinical	[233] [218]
PF-573,228 Pfizer	TKI ATP-C	FAK	Prostate, breast	Preclinical	[219]
GSK2256098 GSK	TKI ATP-C Reversible	FAK, UGT1A1	Solid tumors (ovarian, pancreatic, meningioma, glioblastoma, malignant pleural mesothelioma)	phase I & II	[205-207,215,220] NCT00996671, NCT02523014
NVP-TAC544	TKI ATP-C	FAK	N/A	Preclinical	[221]
VS-4718 (PND-1186) Verastem	TKI ATP-C Reversible	FAK, Pyk2	Solid tumors (pancreas, breast, ovarian), acute myeloid leukemia, B-cell acute lymphoblastic leukemia	phase I	[222]
VS-6062 (PF-562271 and PF271) Verastem	TKI ATP-C Reversible	FAK, CDK2/CyclinE, CDK3/CyclinE, CDK1/CyclinB, Pyk2	Prostate, pancreatic, head and neck	phase I	[208,223]
VS-6063 (Defactinib) Verastem	TKI ATP-C	FAK, Pyk2	NSCLC, pancreatic cancer, ovarian, malignant pleural mesothelioma, hematologic	phase I/Ib & II	[209-211,216,224] NCT02758587, NCT02004028, NCT03875820, NCT03727880, NCT02943317, NCT02913716, NCT02465060, NCT02546531
1H-Pyrrolo(2,3-b) Merk Serono	TKI non-ATP competitive	Hinge region of FAK	N/A	Preclinical	[225]
C4 CureFAKtor	Scaffold inhibitor	FAK /VEGFR-3	Neuroblastoma, pancreatic, breast	Preclinical	[226-228]
Compound R2 (Roslins) CureFAKtor	Scaffold inhibitor	FAK, p53	Colon, reast	Preclinical	[229]
Y11 CureFAKtor	Scaffold inhibitor	FAK Y397 site	Colon, breast	Preclinical	[230]
BI853520	ATP competitive inhibitor	FAK	Malignant pleural mesothelioma, non-hematologic malignancies	Pre&clinical	[213,214,231]}

Table 2: FAK inhibitors with anti-tumor activity in preclinical studies and clinical trials.

Abbreviations: CDK: Cyclin-dependent kinases 1, 2,3; : CureFAKtor: CureFAKtor Pharmaceuticals; GSK: GlaxoSmithKline; FAK: focal adhesion kinase; IGF-IR: insulin-like growth factor 1 (IGF-1) receptor; N/A: data not available; Pyk2: proline-rich tyrosine kinase 2; TKI ATP-C: Kinase inhibitor ATP competitive; UGT1A1: UDP-glucuronosyltransferase 1-1; VEGFR-3: vascular endothelial growth factor receptor 3.

Kinase	PF-228 ^a	
	1 μ M	IC ₅₀
FAK		NM
Pyk2		4
Abl(h)	8	>1000
ACK	29	
AKT	0	
ASK	1	
BTK	11	
c-RAF(h)	0	
CaMKIV(h)	0	
CDK1/cyclinB(h)	77	486
CDK2/cyclinA(h)	50	
CDK2/cyclinE(h)	44	
CDK3/cyclinE(h)	44	
CDK5/p35(h)	0	
CDK6/cyclinD3(h)	15	
CDK7/cyclinH/MAT1(h)	86	197
CHK1	13	
cSRC(h)	1	
EGFR(h)	0	
EphA2	15	
EphB2	11	
Fyn	28	
GSK3 β (h)	64	>1000
IR	27	
IGF-1R	21	
IKK β (h)	56	
JAK3	27	
JNK1 α 1(h)	4	
c-kit	25	
LCK	0	
MAPK1(h)	48	
MEK1(h)	20	
MKK	48	
MKK6	16	
PDGFR- β	2	
P70S6K(h)	31	
PI 3-K γ (h)	0	
PKA(h)	0	
PKB α (h)	13	
PKC α (h)	12	
PKC- θ	36	
ROCK-II(h)	8	

^a Percentage of inhibition.

Table 3: Kinase selectivity profile of PF-228. The inhibitory effect of PF-228 on recombinant CDK1/cycclin B, CDK7/cyclin H, and GSK3 kinase activity was determined. The IC50 values for PF-228 on all kinases were 50–250 times greater than the IC50 for FAK. Adapted from [219].

1.6.2 FAK overexpression and/or activation in human cancers, its frequency and mechanisms

Increased FAK expression or activity has been observed by various methods (Western blot, IHC, Northern blot, quantitative real-time polymeric chain reaction, immunohistochemistry (IHC)) in many human cancers, including lung, head and neck, oral cavity, thyroid, breast, ovarian, prostate, colon, liver, stomach, pancreas, kidney, skin, and bone cancers [234-237]. Increased FAK expression or activity has also been reported in various tumor-derived cancer cell lines [235].

We performed a Pubmed search of studies evaluating FAK protein expression in human cancers by IHC to determine the percentage of cancer samples with increased FAK protein expression. The methods used are described in the legend of Figure 18. Based on this Pubmed search, we found an overexpression of FAK at protein level, as evaluated by IHC, in 80% of pancreatic adenocarcinoma, 72% of neuroblastoma, 70% of ovarian epithelial tumors, and many other cancers, including 52% of NSCLC and 69% of SCLC (**Fig.18**) [191,192,195,197,238-278].

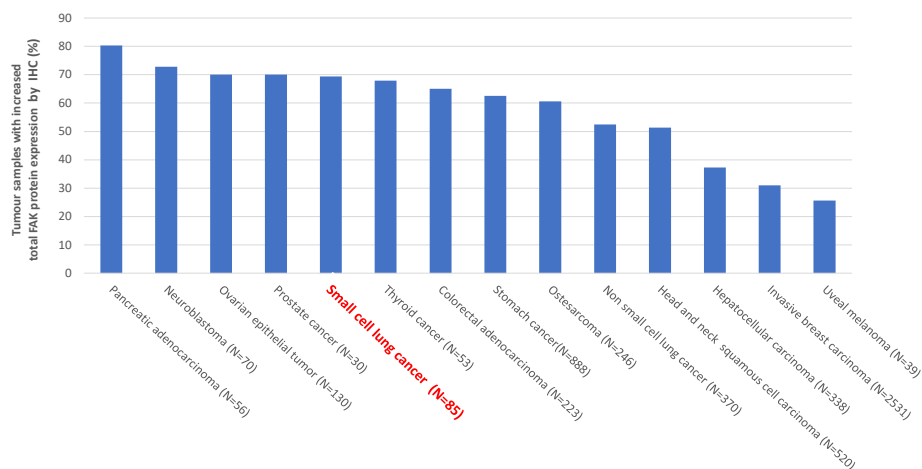


Figure 18: Frequency of focal adhesion kinase (FAK) overexpression at protein level in human solid cancers. A Pubmed search of studies evaluating FAK protein expression in human cancers by immunohistochemistry (IHC) was performed to determine the percentage of cancer samples with increased FAK protein expression.

The following keywords were used in the search strategy: FAK [All Fields] AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND ("immunohistochemistry"[MeSH Terms] OR "immunohistochemistry"[All

Fields)). The results were limited to English language studies. Manual searches of reference articles from applicable studies were performed to identify articles that may have been missed by the computer-assisted search. Abstracts were excluded for cell lines, pre-invasive tumors, if insufficient data to evaluate the methodological quality, absence of tumor total FAK staining, absence of FAK quantification or proportion, absence of proportion of samples overexpressing FAK. Non-eligible trials included ecological studies, case reports, reviews, editorials, and animal trials. This work was conducted in accordance with the PRISMA guidelines.

In The Cancer Genome Atlas (TCGA) database[279], we also found increased FAK expression at mRNA level in several human malignancies, including 51% of uveal melanoma, 49% of ovarian serous cystadenocarcinoma, 41% of liver hepatocellular carcinoma, 34% of breast invasive carcinoma, 23% of lung adenocarcinoma, and 20% of lung squamous cell carcinoma, while it was not reported in SCLC (**Fig.19**).

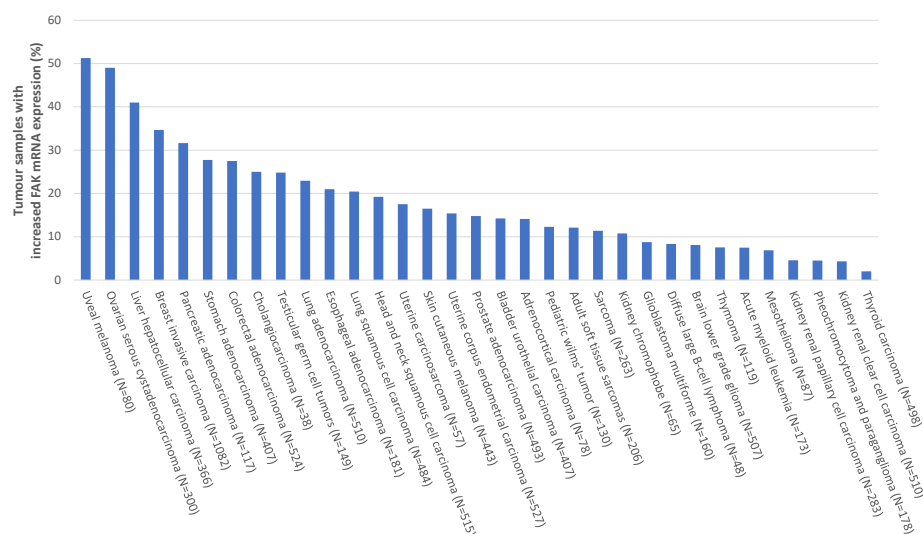


Figure 19: Frequency of increased focal adhesion kinase (FAK) expression at mRNA levels in human cancers. The Cancer Genome Atlas (TCGA) was queried using cbiportal.org to determine the percentage of tumor samples with increased levels of FAK mRNA expression. Search criteria included mRNA expression data (Z-scores for all genes) and tumor datasets with mRNA data. N = number of cancers analysed in the TCGA.

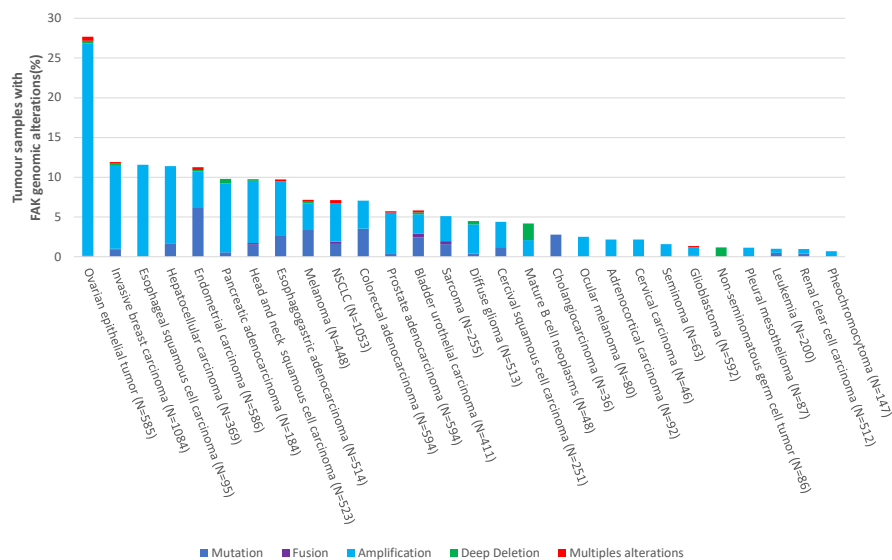


Figure 20: Frequency of focal adhesion kinase (FAK) genomic alterations in human cancers. The Cancer Genome Atlas (TCGA) was queried using cbiportal.org to determine the percentage of samples with FAK genomic alterations (mutations, fusions, amplifications, deep deletions, multiples alterations) in different cancers. Search criteria included PTK2 (FAK). N = number of cancers analysed in the TCGA.

Despite recent progress, underlying mechanisms of FAK overexpression and activation in cancer, especially in SCLC, remain unclear. Putative control mechanisms include gene alterations, transcriptional regulation, post-translational modifications, and interaction with proteases, phosphatases, etc. Among gene alterations, FAK gene amplification within chromosome 8q24.3 and isochromosome formation has been described in many cancers [259,280]. Based on the TCGA database[279], FAK copy number gain is found in 26% of ovarian epithelial tumors, 11.5% of oesophageal squamous cell, 10.4% of invasive breast, 9.7% of hepatocellular carcinoma, and less frequently in other tumors, such as 4.8% of NSCLC (**Fig.20**). In SCLC, specifically, the genomic profiling of tumor samples using genomic comparative hybridization revealed 70 regions of significant copy number gain and 55 regions of significant copy number loss, among which was found an enrichment of 11 genes associated with the focal adhesion pathway, including amplified FAK [199]. FAK gene copy number gain was confirmed by fluorescent in situ hybridization (FISH) in 80% of the SCLC tissues. FAK amplification was also

correlated to increased FAK mRNA expression. At the protein level, evaluated by IHC, FAK was expressed in the cytoplasm of 78/85 (92%) SCLC tissues [195]. In the TCGA database, point mutations with a single-base substitution in FAK gene, resulting in amino acid substitutions in FAK protein, are found in 6.1% of endometrial carcinoma, 3.5% of colorectal adenocarcinoma, 3.3% of melanoma, 2.7% of cholangiocarcinoma, and less frequently in other tumors, including NSCLC (Fig.20), while no data are available in SCLC. Somatic mutations (A1004S point mutations) and splicing variants of FAK have been reported in 7.7% of human NSCLC (**Fig.20**) [281] and have been shown to exhibit increased autophosphorylation and increased sensitivity to FAK kinase inhibitors compared with wild-type FAK in patient-derived xenograft models [281].

FAK gene copy number gains or mutations have not always been correlated with increased FAK expression or activity [190,199,281,282]. Therefore, increased transcription or epigenetic mechanisms may also play a role in increasing FAK expression or activity. Analysis of human FAK gene promoter has identified putative binding sites for transcription factors. NF κ B [283], Argonaute 2 (Ago2) [284], and Nanog [230] are known to activate FAK transcription, while TP53 is a well described repressor of the FAK promoter [285]. Though not explored in SCLC, this last mechanism might be of particular interest in SCLC where TP53 is universally inactivated [148]. According to TCGA, concomitant TP53 mutation and FAK amplification/mutation co-exist in 2% of all cancers. However, these data did not include SCLC samples.

Finally, FAK activation is induced by the engagement of integrins with the ECM or the binding of extracellular growth factors to their receptors. SCLC is well-known to release growth factors such as bombesin, gastrin-related peptide (GRP), HGF, VEGF, TGF- β , HGF, and FGF, which have been shown to activate focal adhesion pathways in several cancers (Fig.16 and 25) [94,95,281,286-292]. Similarly, it has been demonstrated that bombesin, gastrin and bradykinin phosphorylated FAK in SCLC cell lines in vitro [293], suggesting an autocrine and paracrine regulation.

1.6.3 FAK role in proliferation, cell cycle, and survival

FAK activation during cell adhesion protects cells from anoikis, a form of apoptosis induced by cell detachment from ECM, favouring cancer growth and metastasis [294]. FAK is implicated in several pathways contributing to cell survival. Phosphorylated FAK at Tyr397 can bind PIK3R2, leading to the activation of AKT that inhibits apoptosis notably by activating c-JUN kinase, a downstream signal of CAS [204] and the inhibiting receptor-interacting protein (RIP) interaction with the death receptor complex [295].

FAK also induces cell proliferation through the stimulation of cell cycle progression. One of the mechanisms is the formation of FAK/Src complex that allows Src to phosphorylate FAK at Tyr925 and mediate its interaction with Grb2, leading to the activation of the RAS-MAPK signaling pathway [296]. Another mechanism involves the FAK-induced increased expression of cyclin D1 and decreased expression of cycline-dependent kinase (Cdk) inhibitor p21 [297-300]. FAK regulation of cell cycle progression is also mediated by other cell cycle regulators such as cyclin E, Cdk inhibitor p27, and Skp2 [301-304]. Moreover, FAK is important for tumor cell-induced remodeling of the tumor matrix, which produces a rigid microenvironment and facilitates cell proliferation [305].

1.6.4 FAK role according to cellular compartment

FAK is activated by integrins, G protein-coupled receptors (GPCRs), cytokine receptors, and growth factor receptors, such as HER-1 or HER-2, or VEGFR-3, at focal adhesions at the cell surface (**Fig.22, 23, and 24**). Through its kinase activity, FAK activates downstream signaling proteins, such as the Src kinase. In the absence of receptor signals, FAK accumulates in the cytoplasm (**Fig.21 and 22**), where, through a kinase-independent mechanism, FAK sequesters several proteins such as protein kinase RIP (receptor-interacting protein) and TP53 [306]. FAK and TP53 both shuttle between the cytoplasm and the nucleus to mediate prosurvival or proapoptotic signaling. Whereas TP53 stimulates the transcription of the gene encoding p21, which causes cell cycle arrest, TP53 binds to the promoter region of FAK to inhibit its transcription. In the nucleus, FAK through its FERM domain acts as a scaffold protein to bind to TP53 and the E3 ubiquitin ligase Mdm2, causing the ubiquitination and degradation of TP53, therefore preventing transcription of the gene encoding p21, which causes cell cycle arrest. TP53 also binds to the promoter region of FAK to inhibit its transcription (**Fig.22**) [190,306]. FAK may also sequester other proapoptotic proteins in the nucleus and cytoplasm in a kinase-independent manner to promote cell survival.

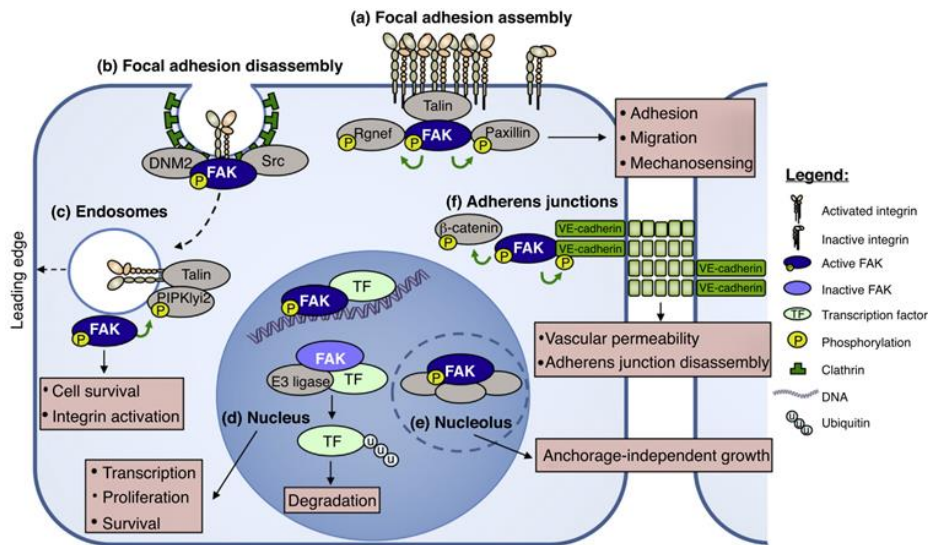


Figure 21: FAK functions throughout the cell according to cellular localization (from [203].) FAK functions in both kinase-dependent and independent manners. Activated FAK goes to the nucleus and potentially regulates gene expression to affect cancer progression. Nuclear FAK functions as a scaffold for TP53 and MDM2 in a kinase-independent manner, increasing TP53 polyubiquitylation and degradation, thereby promoting cell survival[306,307].

- (a) Activated FAK phosphorylates Rgnet and paxillin, promoting the assembly of focal adhesions. FAK at adhesions regulates cell adhesion, migration, and mechano-sensing.
- (b) FAK binds dynamin-2 (DNM2), triggering focal adhesion disassembly and integrin endocytosis.
- (c) FAK binds to and is activated at endosomes. FAK phosphorylation of PIPKIγ2 results in talin recruitment, which in turn maintains the active conformation of endosome-associated integrins. FAK-endosome signaling facilitates the polarized reassembly of activated integrins at the leading edge of migrating cells. FAK at endosomes facilitates integrin activation and cell survival.
- (d) In the nucleus, activated FAK binds to transcription factors (TFs) to modulate gene expression. Inactive FAK in the nucleus functions as a

scaffold to facilitate transcription factor turnover via enhanced ubiquitination by complexing with different E3 ligases.

- (e) In the nucleolus, activated FAK complexes with proteins important in promoting stem cell-like phenotypes. FAK in the nucleus and nucleolus alters transcription, survival, and anchorage-independent cell growth.
- (f) Active FAK localizes to adherens junctions (AJs) and directly binds VE-cadherin in response to vascular endothelial growth factor stimulation of endothelial cells. FAK phosphorylates b-catenin and VE-cadherin, triggering AJ disassembly.

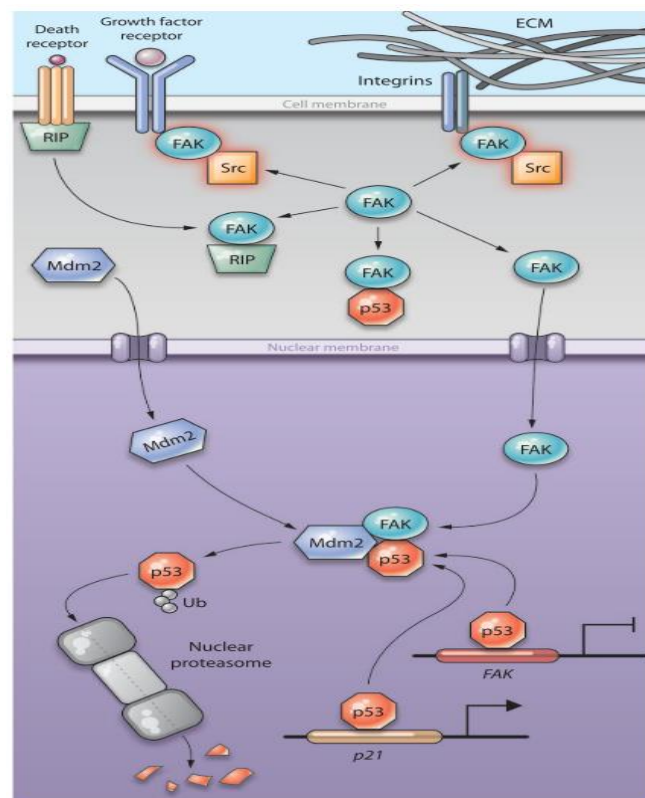


Figure 22: FAK and TP53 interactions at the cytoplasmic and nucleus compartments (from [306]).

1.6.5 FAK role in adhesion, migration, and invasion

FAK induces morphological changes in cells, including the formation of podosomes or invadopodia, contributing to cell migration [308-310]. Moreover, cancer cells overexpressing FAK are able to invade tissues [311]. By promoting cell migration and invasion, FAK overexpression contributes to the metastatic phenotype of cancer cells.

Cell migration is a complex process, consisting of several coordinated events, including protrusion of the leading edge, adhesion of the leading edge to the substrate [312], translocation of the cell body, and release of the trailing edge [313]. Thus, a strict regulation of tension at specific times and in specific areas of the cell is required for cell migration [314,315], where FAK plays an important role by sensing the mechanical forces generated in or exerted on cells [316], and modulating cell responses to environmental stimuli (**Fig.21 and 23**). Once activated by integrins, G protein-coupled receptors ligands or growth factors, FAK is autophosphorylated at Tyr397 and activates proteins such as Src, p130CAS, paxillin and PIK3R2 (**Fig.16 and 21**) [317] to regulate adhesion turnover at the cell front, a process central to migration [317-321]. FAK is indeed required for the organization of the leading edge in migrating cells [322]. The formation of a complex between FAK and Src, leading to the phosphorylation of the adaptor molecule CAS by the FAK/Src complex [323-327], is one of the best characterized downstream signaling pathways that mediate FAK-stimulated cell migration. A second mechanism involves FAK interaction with PIK3 and an adaptor molecule, Grb7 [328,329]. A third mechanism involves the modulation of the assembly and disassembly of actin cytoskeleton through the effect of FAK on the Rho family GTPases. Among the Rho family GTPases, FAK/Src signaling has in particular been implicated in regulating the activities of Rac1 and RhoA (**Fig.23**).

Besides its role in cell migration, FAK promotes invasion in normal and cancer cells by various mechanisms. In one of them, FAK promotes the formation of the Src-CAS-Crk-Dock180 complex which activates Rac1 and JNK, and leads to increased expression or activity of metalloproteinases 2 (MMP2) and 9 (MMP9) [310]. MMPs are concentrated and activated at actin-rich cell / ECM contacts termed podosomes or invadopodia, which are distinct from focal adhesion. In another mechanism, FAK cooperates with Src to disrupt the E-cadherin-based intercellular adherens junctions [330], contributing to EMT and therefore to the invasive phenotype of metastatic carcinomas through increased cell migration and remodelling of the ECM microenvironment [331-333].

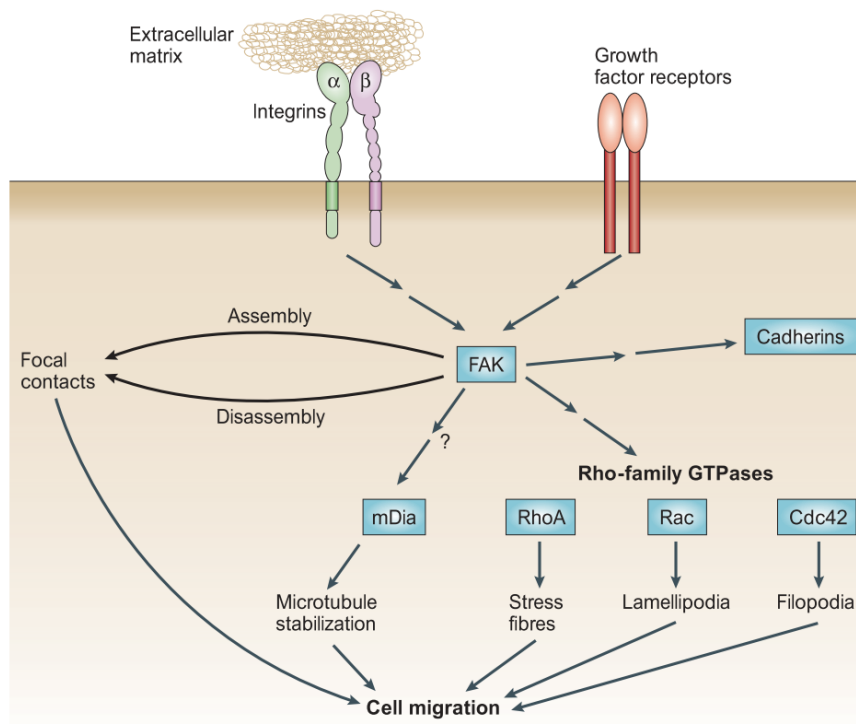


Figure 23: Connexion between FAK and Rho-family GTPases at the focal adhesion complex (from [318]).

1.6.6 FAK in epithelial to mesenchymal transition

Through epithelial-to-mesenchymal transition (EMT), cancer cells acquire a more motile phenotype, promoting invasion, metastasis, but also conferring resistance to chemotherapies and targeted therapies. Epithelial cancers undergoing EMT acquire transient mesenchymal features like vimentin and N-cadherin, associated with loss of epithelial markers E-cadherin and β -catenin [334]. EMT is correlated with poor outcomes in SCLC [335], such as in many other cancers. Identified mechanisms inducing EMT in SCLC include inactive Notch signaling [336], activated MET receptor signaling through hepatocyte growth factor [335], and activated TGF β / Akt signaling [337].

While FAK-mediated EMT has not yet been explored in SCLC, its important role has been demonstrated in other cancers and non-malignant cells [338-341]. Impaired FAK functions lead to a defective mesenchymal phenotype during EMT. Hence, upon TGF β -induced EMT, hepatocyte cell lines transduced with FRNK, a genomic method to inhibit FAK, underwent an incomplete mesenchymal transition, exhibiting a lack of mesenchymal markers MMP9 and fibronectin and a persistence of

membrane-bound E-cadherin [338]. Mammary tumor cells with deficient FAK scaffolding function due to Pro 878/881 mutation displayed also incomplete mesenchymal phenotype with increased E-cadherin and decreased N-cadherin, Vimentin and fibronectin in a mice model [339]. It was associated with decreased metastasis potential and decreased expression of EMT-inducing gene Snail 1 [339]. Similar reduction of Snail 1 in embryonic FAK-null cells has been associated with inability to display mesenchymal cell characteristics, while reexpression of FAK restored mesenchymal phenotype and Snail 1 level through PI3K/Akt signaling [340]. In ovarian cancer, FAK controls EMT by upregulating transcription factor KLF8 via PI3K/Akt pathway [341]. It has been shown that transcription factors Snail 1 and KLF8 repress E-cadherin expression, promoting EMT in various normal and malignant cells [342-344]. Inhibition of FAK by a genetic or a pharmacologic method decreased EMT features and aggressiveness in colorectal carcinoma cell lines [345,346] and triple negative breast cancer cell lines in vitro [347], but not in NSCLC cell lines in vitro [348].

1.6.7 FAK-mediated angiogenesis and vascular permeability

The role of angiogenesis and vascular permeability is fundamental to the progression of cancer from localized to advanced-stage disease [349-351]. Tumors induce local generation (vasculogenesis) and subsequent growth (angiogenesis) of new vasculature that facilitates the supply of oxygen and nutrients to cancer cells [350]. Moreover, it has been shown that SCLC cells in tumors or in the blood harbours markers of vascular mimicry, including expression of vascular endothelial cadherin (VE-Cadherin). Vascular mimicry could therefore supply nutrient and oxygen required for the expansion of SCLC cells [352]. Furthermore, several molecules have been shown to promote angiogenesis and/or vascular permeability, for instance vascular endothelial growth factor (VEGF), hypoxia inducible factor (HIF), fibroblast growth factor (FGF), transforming growth factor beta (TGF- β), hepatocyte growth factor (HGF), tumor necrosis factor- α , angiogenin, ephrins, and angiopoietins [90,286,349,351,353]. SCLC produces many of these pro-angiogenic factors, including VEGF, TGF- β , HGF, and FGF (**Fig.24**) [94,95,289-292]. Moreover, SCLC displays a higher vascularisation compared to other tumours. Both high tumor vascularisation and high VEGF expression are associated with poor outcome in SCLC [94,96]. High VEGF expression has also been correlated to an increased risk of extensive disease [96]. This stressed out the strong connexion between angiogenesis, vascular permeability, and the development of metastases in SCLC, which is a highly metastatic disease with a high prevalence of circulating tumour cells (CTCs) (**Fig.24**) [20,45,91-93]. Several clinical trials have demonstrated that antiangiogenic agents such as bevacizumab, pazopanib, and sunitinib increased progression-free survival (PFS) in SCLC despite they failed to show a significant benefit in terms of OS [114,122,354,355]. These results are probably related to the

absence of relevant biomarkers to select patients who might better benefit from antiangiogenic agents.

FAK has a crucial role in angiogenesis and vascular permeability, as demonstrated by the vascular defects in FAK double knockout mice, resulting from the inability of FAK-deficient endothelial cells to organise themselves into vascular networks [356]. Also, overexpression of FAK in vascular endothelial cells promotes angiogenesis. For instance, the number of vessels in the granulation tissue of healing wound was significantly increased in transgenic mice (overexpressing FAK) compared to wild-type control mice [357]. Additionally, VEGF-induced vascular permeability is mediated by FAK signaling (**Fig.24**), with inhibition of FAK activity in endothelial cells suppressing VEGF-stimulated vascular permeability [358]. It has been shown that FAK triggering by VEGF is abrogated by FAK inhibitors, which decrease vascular permeability and tumor vasculature, preventing tumor growth, metastasis, and immunosuppressive tumor infiltration by cells such as tumor macrophages and T regulatory cells (**Fig.24**) [278,358-362]. Additionally, it has been shown that withdrawal of antiangiogenic therapy results in accelerated tumor growth and that this tumor rebound is mediated by FAK activation, which increases angiogenesis and platelet infiltration (**Fig.24**) [363]. Interestingly, FAK inhibition prevents tumor rebound after cessation of antiangiogenic therapy through inhibition of tumor angiogenesis, platelet-induced tumor cell proliferation, and vascular leakage (**Fig.24**) [363-366]. Of note, there is no data on the role of FAK in angiogenesis and vascular permeability in SCLC.

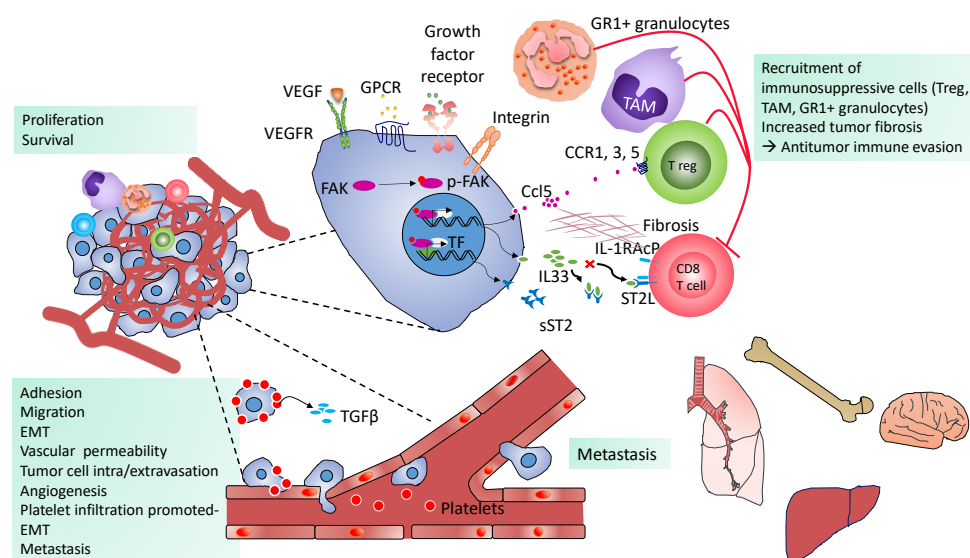


Figure 24: Pro-tumoral functions of FAK. (A). FAK is triggered off by integrins, G protein-coupled receptors (GPCR), growth factor receptors, and vascular endothelial growth factor receptor (VEGFR). Activated FAK promotes cell proliferation and survival. FAK also contributes to tumor progression and metastasis via cell adhesion, migration, and promotion of epithelial to mesenchymal transition (EMT). Transient contact between platelets and tumor cells induces TGF β production by the platelets, which promotes EMT-like transformation and invasive behaviour. In endothelial cells (EC), FAK drives angiogenesis, increases vascular permeability, and regulates platelet extravasation; this facilitates intravasation or extravasation of tumor cells, leading to metastasis. Additionally, FAK induces a tumor protective fibrotic and immunosuppressive tumor microenvironment that promotes antitumor immune evasion. Indeed, FAK induces cytokines (short soluble (sST2), IL33, Ccl5), which lead to the recruitment of immunosuppressive cells, such as regulatory T cells (Treg), tumor-associated macrophages (TAM), and GR1+ granulocytes, as well as to increased tumor fibrosis.

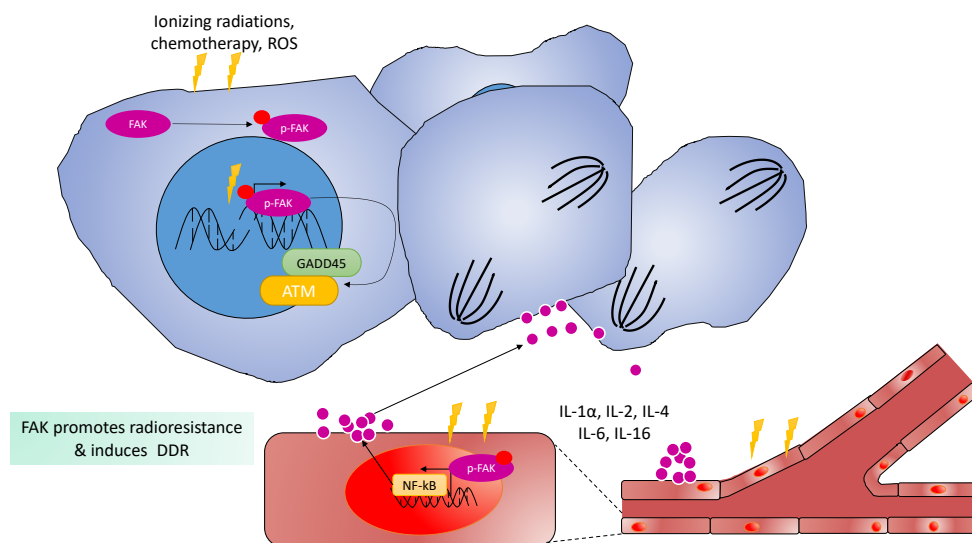


Figure 25: Ionizing radiations, chemotherapy, and reactive oxygen species (ROS) increase DNA damage and activate FAK in tumor cells. Activated FAK favors the expression of DNA damage repair (DDR) genes such as Growth Arrest and DNA Damage-inducible 45 (GADD45), Ataxia Telangiectasia Mutated (ATM) genes, and Ataxia Telangiectasia and Rad3-related (ATR) genes which play an important role in resistance to

drug and radiation. Additionally, in endothelial cells (EC), ionizing radiations activate FAK and NF- κ B, which induces the production of various cytokines (IL-1 α , IL-2, IL-4, IL-6, IL-16) activating the proliferation of tumor cells. Abbreviations used in the figure and not described in the legend: IL-1RAcP: interleukin-1 receptor accessory protein, ST2L: longer membrane bound form.

1.6.8 FAK and DNA damage repair

Exposure to endogenous and exogenous carcinogens (e.g. reactive oxygen species, UV light, tobacco smoking, ionizing radiation, platinum chemotherapy) generates DNA damage both in normal and cancer cells [367]. Signaling pathways activated by cells to sense and repair DNA damage, preventing genomic instability, are known as DNA damage repair (DDR) [368,369]. DNA-damaging chemotherapy and radiotherapy are used alone or in combination in the treatment of ES- and LS-SCLC, respectively. SCLC tumors are initially responsive to the treatment but development of early resistance limits outcomes. Objective response rates of 80-90% are achieved in LS-SCLC treated by concurrent radiochemotherapy [49,50] and of 60-70% in ES-SCLC treated by platinum-based chemotherapy [21,54], but the median OS is only 25-30 months in LS-SCLC and 12 months in ES-SCLC [50,62,147]. Understanding the underlying mechanisms of acquired or intrinsic radioresistance and/or chemoresistance is important for the improvement of SCLC survival.

It has been shown that DDR genes and proteins are more highly expressed and activated in SCLC compared to NSCLC and that blocking these DDR pathways has antitumoral activity both in preclinical [370] and clinical [371] studies of different types of cancer. In SCLC, the association of the PARP inhibitor olaparib and the anti-PD-L1 ICI durvalumab in a phase II trial did not meet efficacy criteria but revealed that responses were observed only in tumors with an inflamed phenotype on tissue biopsies at baseline, suggesting that the tumor microenvironment (inflammation phenotype) is a potential predictive biomarker [372]. Another phase II trial with the PARP inhibitor veliparib combined or not to the chemotherapy agent temozolomide in recurrent SCLC showed an improved overall response rate without improvement of PFS and OS in the combination arm, but patients with SLNF11 (inhibitor of DNA replication)-positive tumors treated with the association had a significantly improved PFS and OS, suggesting that SLNF11 is a predictive biomarker [371].

Interestingly, FAK promotes DDR by promoting the transcription of genes favoring DDR such as growth arrest and DNA damage-inducible 45 (GADD45), ataxia telangiectasia mutated (ATM), and ataxia telangiectasia and Rad3-related (ATR) **(Fig.25)** [373,374]. Furthermore, FAK inhibition promotes the hyperactivation of downstream targets of ATM/ATR such as checkpoint kinase 2 (CHK2) [374]. In in

vitro and in vivo preclinical models of NSCLC harbouring KRAS mutations, ionizing radiation leads to FAK activation (Tyr397 phosphorylation), which persists for several hours, while inhibition of FAK activity leads to an inherent loss of DNA repair capacity and to radiosensitizing effects that promote the therapeutic effect of ionizing radiation [373-375]. Similarly, FAK has also been shown to regulate human ductal carcinoma in situ (DCIS) cancer stem cells (CSC) activity and response to radiotherapy [376]. While CSC harbor ability to avoid or efficiently repair DNA damage from radiotherapy and chemotherapy, which play a role in disease recurrence, inhibition of FAK activity potentiated the effect of irradiation in DCIS CSC [376]. Finally, it has been shown that FAK regulates tumor resistance to DNA-damaging therapies through NF- κ B activation and subsequent cytokine production. Interestingly, FAK inhibition sensitizes tumour cells to chemotherapy by suppressing NF- κ B activation and subsequent cytokine production (e.g. IL-1 α , IL-2, IL-4, IL-6, IL-16...) (**Fig.25**) [377]. Even though no data are available on the role of FAK in DDR in SCLC, based on these findings in other cancers, we hypothesize that FAK TKI may also be used in SCLC to improve the efficiency of chemotherapy and/or radiotherapy by impairing DDR and/or increasing DNA damage.

1.6.9 FAK and radioresistance

Radiotherapy associated with chemotherapy remains the cornerstone of LS-SCLC treatment despite frequent emergence of resistance and cancer recurrence. Understanding the underlying mechanisms of acquired or intrinsic radioresistance is important for the improvement of SCLC survival. Several mechanisms have been involved in tumor radioresistance. Among those, adhesion molecules have a key role against radio-induced apoptosis, in a phenomenon called “cell adhesion-mediated resistance” [378-382]. In SCLC, the spontaneous transformation of cell lines in culture since several months into more adherent and radioresistant sublines highlights this mechanism [383,384]. FAK, as a key player in the focal adhesion pathway, mediates this anti-apoptotic action against ionizing radiation. Hence, irradiation of a promyelocytic leukemia cell line overexpressing FAK induced less DNA fragmentation and cell death than in control cells [385]. Accordingly, a proteomic analysis showed that FAK expression was strongly correlated with radioresistance in a large panel of head and neck (HN) squamous cell carcinoma (SCC) cell lines [386]. Moreover, ionizing radiation upregulated in vitro expression and activation of FAK in breast cancer, glioblastoma, and lung cancer cell lines, leading to acquired radioresistance [375,387]. Inhibition of FAK using genetic (FAK shRNA transduction) or pharmacological (FAK TKI) methods radiosensitized KRAS-mutated NSCLC, significantly decreasing radiation survival in vitro and in vivo [374]. Similar results have been reported in HNSCC [386,388,389] and in pancreatic carcinoma [378].

Several FAK downstream signaling pathways have been involved in FAK-mediated survival after ionizing radiation. In a promyelocytic leukemia cell line overexpressing FAK, the Phosphoinositide 3-kinase (PI-3K)-Akt survival pathway is constitutively activated. Moreover, FAK prevents radiation-induced cell death by downregulating the mediator of apoptosis Caspase 8 and by upregulating inhibitor-of-apoptosis proteins like c-IAP and XIAP [385]. Concomitant activation of NF- κ B has also been reported [385]. FAK inhibition radiosensitized HNSCC cell lines in vitro through MAPK and Akt signaling dephosphorylation [389]. In spontaneous radioresistant SCLC cell lines, constitutive activation of Akt and MAPK pathways and increased level of active NF- κ B are similarly observed [384]. FAK interaction with JNK1 also has an important role for radioresistance in pancreatic carcinoma cell lines [378] and in HNSCC cell lines [390].

Even though not explored in vivo yet, FAK inhibition may be a useful approach to improve radiotherapy efficacy in SCLC. Cautions are nevertheless mandatory since the effects of FAK inhibition on radiosensitivity depend on the tumor type. While FAK pharmacological inhibition combined with radiation radiosensitized HNSCC, it did not show any additional effect in vitro on ionizing radiation lethality in non-Kras mutated NSCLC, colorectal carcinoma, and pancreatic carcinoma cell lines [388].

1.6.10 Regulation of cancer stem cells

CSC hypothesis emerged over the last 150 years [391] and progressively replaced the clonal evolution theory of carcinogenesis [392]. This model postulates that a tumor arises from a subpopulation of pluripotent cells capable of extensive self-renewal and resistance to ionizing radiation and chemotherapies. Altogether, these aggressive subtypes of malignant cells are presumed to be responsible for recurrence after treatment [393]. The existence of CSCs in SCLC has been demonstrated in cell lines and primary tumors [394-398], participating to therapy resistance and rapid recurrence of SCLC [397,399,400]. CSCs have been identified in SCLC based on the analysis of cell surface markers and functional properties such as the capacity to exclude Hoechst dye, to form tumorspheres, and to initiate tumor after xenotransplantation in mice, mirroring their tumorigenicity. In SCLC, common markers used of study CSCs are CD133, ALDH1 and pluripotency-related gene Nanog, Oct3/4, and Sox 2 among others (reviewed in [401]). Some of these markers have been correlated with poor prognosis [402-404]

While not explored yet in SCLC, critical role of FAK in CSCs maintenance has been described in several cancers. It has been demonstrated that the CSC marker Nanog upregulates FAK that in turn phosphorylates Nanog in CRC cell lines [230]. Upregulation and activation of FAK has also been observed in presence of Oct 3/4-surexpressing glioblastoma primary cell cultures [405]. CD133, another CSC marker, enhanced cell migration through Src-FAK signaling activation [406]. Furthermore, a

strong influence of ECM in sustaining CSCs through FAK signaling has been demonstrated in pancreatic ductal adenocarcinoma, colorectal cancer, and breast cancer [407-409]. Additional proof of FAK implication in CSCs is that several drugs effective against CSCs act through FAK inhibition [410-413]. Several studies have demonstrated that FAK inhibition preferentially eliminates CSCs pool in vivo and in vitro in various cancers [376,407,414-419]. In pancreatic ductal adenocarcinoma, FAK inhibition with a TKI or shRNA impacted tumor-initiating potential, self-renewal, and metastasis, and improved response to chemotherapy via CSCs regulation both in vitro and in vivo [407]. FAK TKI decreased more efficiently proliferation and survival of the CSCs subpopulation in malignant mesothelioma [414,415], and its administration after chemotherapy improved disease-free survival in a mouse model [415]. In breast cancer, similar effects of FAK inhibition were obtained on the CSCs pool in vivo and in vitro [376,416,417] and on the duration of response after chemotherapy [417]. In mice, FAK knockout prevented induction and growth of skin SCC, suggesting decreased capacity of CSCs generation and maintenance [418]. Finally, colorectal CSCs were preferentially targeted by FAK TKI in vitro in human cell lines compared to non-CSCs [419]. Both FAK kinase dependent and independent functions have been implicated in CSCs maintenance and regulation in breast cancer [420]. Interestingly, FAK inhibition suppressed β -catenin activation, confirming a crosstalk between FAK and Wnt/ β -catenin pathway [376,417]. Given the poor response and rapid recurrence of SCLC after chemotherapy, we hypothesize that combination of FAK TKI with conventional treatment might be a pertinent therapeutic strategy.

1.6.11 FAK in tumor immune escape

ICIs induced remarkable improvements in tumor response and OS in many types of solid tumors, including NSCLC, both in pretreated and treatment-naïve advanced-stage disease [39,40,42,43,421]. The most robust objective response rates to ICIs have been shown in tumors with high PD-L1 expression, even though PD-L1 remains an imperfect biomarker [422]. As opposed to NSCLC, there is a lack of correlation between PD-L1 expression and response to ICIs in SCLC [423] and the efficacy of ICIs in terms of response rates and OS is limited in SCLC patients [62]. The IMpower133 trial, comparing carboplatin plus etoposide with or without atezolizumab, a PD-L1 inhibitor, in first-line treatment of patients with ES-SCLC, showed only a 2-month improvement in OS in the atezolizumab arm. [62]. Similar results were obtained with durvalumab instead of atezolizumab, another PD-L1 inhibitor, in the CASPIAN trial [63]. Nevertheless, it was the first time since several decades that an improved survival was obtained in ES-SCLC. Based on these two trials, chemotherapy combined with atezolizumab or durvalumab recently became the new standard of care in the first-line treatment of ES-SCLC.

SCLC displays high capacities to escape immune surveillance through several processes. Among those, it has been demonstrated that SCLC cell lines have the capacity to induce regulatory T cell (Tregs) in vitro [424]. This is an important mechanism, as Tregs infiltration in SCLC biopsies has been correlated with poor survival of patients [424]. Interestingly, a study recently demonstrated a role for FAK in controlling Treg levels in cutaneous and pancreatic tumors [278,425]. In skin SCC, FAK drove the recruitment and expansion of Tregs within the tumor, subsequently impairing the antitumor response of CD8+ cytotoxic T lymphocytes [425]. Xenograft of FAK-deficient SCC in mice failed to durably develop and exhibit a CD8+ T cells-dependant tumor regression within 21 days, as opposed to FAK-wild type tumor cells [425]. Pharmacological inhibition of FAK in a skin SCC mouse model decreased the levels of Tregs and increased those of CD8+, confirming the key role of FAK in immune escape [425]. Similar results were observed in pancreatic ductal adenocarcinoma and colorectal cancer, where association of FAK inhibitors with immunotherapy markedly improved survival of the mice [278,426]. Mechanistically, FAK controls Tregs infiltration in skin SCC through transcription of chemokines and cytokines via its nuclear interaction with transcription factors and regulators [362,425]. Among those increased genes, Ccl1, Ccl5, and TGFβ2 have been involved in Tregs conversion and recruitment in various cancers [427-432].

Additionally, an immunosuppressive role of myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAM) promoting tumor development by impairing antitumor immunity has been described in various cancers [433]. In SCLC, peripheral MDSC count has been correlated with poor prognosis [434] and tumor progression induced by TAM has been demonstrated in vitro [435]. Interestingly, FAK TKI also decreased tumor-infiltrating immunosuppressive cells in pancreatic [278,360] and breast cancers [436]. In SCC, FAK TKI promoted tumor control by reducing tumor-infiltrating regulatory T cells and increasing T CD8+ T cells [425]. Furthermore, it has been shown that FAK promotes expression of interleukin-33 (IL-33), soluble secreted form of the IL-33 receptor, called soluble ST2 (sST2), and chemokine CCL5 (CCL5), which mediate FAK kinase-dependent antitumor immune evasion of SCC cells [362].

Even though the role of FAK in immune tumor escape has not been proven yet in SCLC, these studies raise the hope to improve outcome of patients through the association of FAK TKI with immunotherapy or conventional chemotherapy. In advanced pancreatic cancer, mesothelioma, and NSCLC, a clinical trial evaluating the association of FAK (VS6063) and PD-1 (pembrolizumab) inhibitors is ongoing (NCT02758587).

1.6.12 Interplay between FAK and Rho-family GTPases at the focal adhesion complex

RAC1 is a member of the Rho GTPase family proteins, which include RHO, RAC1, and CDC42 [437]. Rho GTPases are crucial regulators of cytoskeletal dynamics through the organization of the actin cytoskeleton [437-439]. RHO, RAC1, and CDC42 switch between GTP-bound (active) state to GDP-bound (inactive) state (**Fig.23 and 26**). The active state is mediated by guanine nucleotide exchange factors (GEFs). The inactive state is promoted by GTPase activating proteins (GAPs), which activate the endogenous GTP hydrolyzing activity of Rho GTPases [439,440]. When activated, Rho GTPases interact with downstream effectors that lead an intracellular signaling cascade (**Fig.26**) [439].

It has been shown that Rho GTPases mediated several processes that underlie malignant transformation, including tumorigenesis, cell cycle progression, cell migration, angiogenesis, invasion, and metastasis (**Fig.26**) [437-442]. Furthermore, both RAC1 and FAK are localized in the focal adhesion complex and play important role in cell motility. It has been shown that RAC is required in the formation of new focal adhesions complexes (**Fig.23 and 26**) [443]. Additionally, cell adhesion to the ECM, which involves interaction between FAK and integrins, activates RAC [444]. Rho GTPases direct local actin assembly into stress fibres, lamellipodia, and filopodia. FAK can influence the activity of Rho-family GTPases through a direct interaction with, or phosphorylation of, protein activators or inhibitors of Rho GTPases [190,318]. It has been shown that Rac1 tyrosine residue 64 is a substrate of both Src and FAK that exerts a downward regulatory effect in RAC1 focal adhesions targeting and in cell spreading [445].

High expression of RAC1 was shown to be associated with poor outcome in several human cancers, such as breast, colorectal cancers, and leukemia [442,446]. RAC1 overexpression and activation has been reported in several cancers including melanoma, NSCLC, breast and gastric cancer [447]. Mechanisms that lead to RAC1 activation include RAC1 mutations (such as RAC1 P29S in melanoma) [448] and missense mutations in the ubiquitin ligase HACE1 (HECT-domain and Ankyrin-repeat Containing E3 ubiquitin protein ligase 1) that leads to defective Rac degradation [449,450]. RAC-GEF hyperactivation and RAC-GAP downregulation has also been reported as a mechanism of RAC1 activation in melanoma, ovarian, prostate, breast, lung, and colon cancer [440,451].

The role of RAC1 in enhancing nucleotide metabolism and inducing chemoresistance has been reported in multiple human cancers [446,452]. For instance, RAC1 upregulates the R5P (Ribose 5-phosphate) synthesis and

nucleoside metabolism, thus promoting the repair of DNA damage caused by chemotherapy agents, and inducing chemoresistance of breast cancer cells [453].

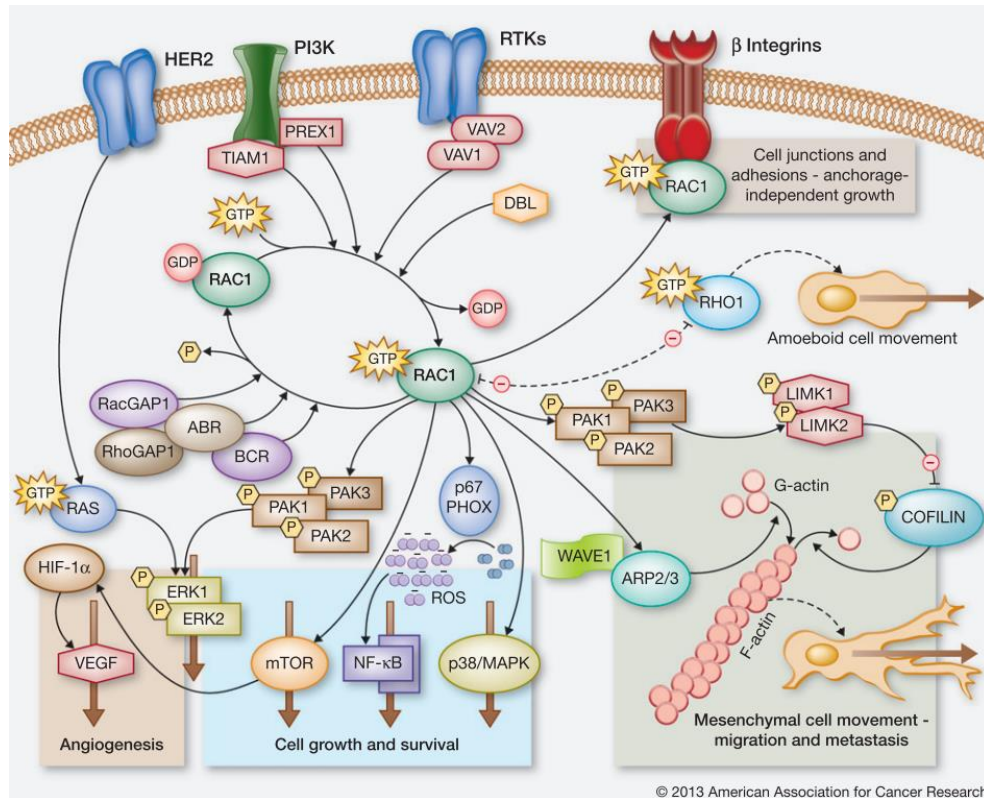


Figure 26: RAC signaling pathways and effector functions. RAC1 cycle between an inactive GDP-bound form and an active GTP-bound form. Emphasis is given to pathways known to affect tumor related angiogenesis, cell survival, and metastasis (from [438]). Rho GTPases are also essential for the cytoskeletal changes underlying cell motility and invasion, which allow cancer cells to migrate away from the primary tumor and invade surrounding and later distant tissues, ultimately developing metastasis.

1.6.13 Prognostic and predictive value of FAK alterations

FAK genetic alterations reported in the Cancer Cohort of TCGA project were correlated with PFS (Figure 5), and FAK overexpression at mRNA and protein levels were correlated with poor OS in several cancers [190,282]. FAK protein overexpression was associated with worse OS in gastric cancer (HR=2.646, 95% CI:1.743–4.017, p=0.000), hepatocellular cancer (HR=1.788, 95% CI: 1.228-2.602, p=0.002), ovarian cancer (HR=1.815, 95% CI: 1.193-2.762, p=0.005), endometrial

cancer (HR=4.149, 95% CI: 2.832-6.079, p=0.000), gliomas (HR=2.650, 95% CI: 1.205-5.829, p=0.015), and squamous cell head and neck and digestive cancers (HR=1.696, 95% CI: 1.030-2.793, p=0.038) [282].

In SCLC, no correlation was found between total FAK expression evaluated by IHC on 85 SCLC tissues and SCLC disease stage, response to therapy, PFS, or OS [195]. Similarly, total FAK and phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence in tissues from 105 SCLC and 95 NSCLC patients did not correlate with PFS or OS [454]. However, even in the absence of a prognostic value, a predictive value of response to FAK TKIs cannot be ruled out. Several clinical trials have evaluated FAK TKI in patients suffering from various advanced-stage cancers, showing antitumor activity (up to 33% objective response rates) and safety [206,207,209,211], while they did not use biomarkers such as FAK or phospho-FAK expression to identify patients likely to respond to FAK TKI. It would be interesting for future clinical trials evaluating FAK TKI to prospectively test total FAK and activated FAK expression as potential predictive biomarkers of response to FAK TKI.

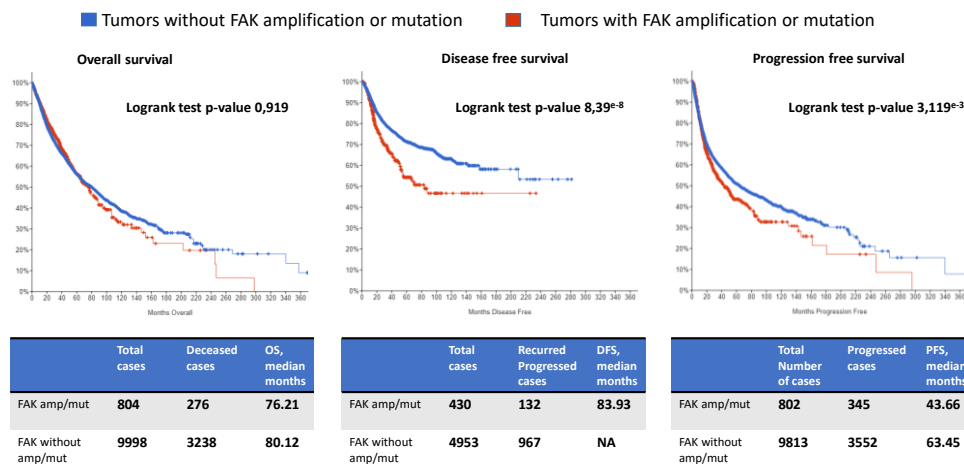


Figure 27: Association of focal adhesion kinase (FAK) amplification with survival. Kaplan-Meier overall survival, disease free and progression-free survival analysis of patients with versus without FAK amplification or mutation in their tumors (many different cancers included) in The Cancer Genome Atlas (TCGA) database (<http://www.cbioportal.org/>).

1.6.14 Conclusions and therapeutic perspectives

In this review, we have presented an overview of the role of FAK in cancer development and progression, through its functions in cell growth, survival,

adhesion, spreading, migration, invasion, angiogenesis, DNA damage repair, radioresistance, and regulation of CSC. This constitutes the biological rationale to consider FAK as a potential therapeutic target in SCLC. Association of FAK inhibitors with standard therapies of SCLC - platinum-based chemotherapy, radiochemotherapy, or immunotherapy may have synergistic effects and improve outcomes of SCLC patients. We hope that the development of specific FAK inhibitors will have clinical translational significance in SCLC by targeting, among others, antitumor immunity, angiogenesis, EMT, regulation of CSC, DDR, and therapy resistance, including radioresistance, which are crucial in SCLC biology.

2 BACKGROUND AND GOALS OF THE THESIS RESEARCH

This work started on the basis of a former study performed by Prof. Ocak (promoter of the present project) et al. who performed array comparative genomic hybridization (CGH) on DNA extracted from 46 SCLC tissues, using a 2464 BAC clone array [199]. Genomic profiling of tumor and sex-matched control DNA allowed the identification of 70 regions of amplification (including 329 genes) and 55 regions of deletion (including 99 genes). Using molecular pathway analysis, they found a strong enrichment in these regions of copy number alterations for eight genes associated with the neuroactive ligand-receptor pathway and 11 genes associated with the focal adhesion pathway. These findings were verified at genomic, gene expression, and protein levels. They decided to focus on the focal adhesion pathway and, among the genes represented in it, on the amplified FAK. FAK is a non-receptor tyrosine kinase localized at sites of focal adhesions and plays an important role in signaling pathways initiated by integrin-, G-protein-coupled and growth factor-stimuli [293,455]. It is overexpressed in many cancers and possibly contributes to cancer progression through an important role in cell adhesion, spreading, migration, invasion, survival, and anchorage-independent growth [190,250,456,457]. Since FAK has been poorly studied in SCLC, we selected it as a candidate pathway to be investigated further.

FAK was commonly expressed in primary SCLC tumors [195], and constitutively phosphorylated in SCLC cell lines [199]. Treatment with a FAK inhibitor, PF-573,228 (PF-228), decreased FAK phosphorylation at FAK (Y397), inhibited adhesion, and changed the phenotype of the cells. While cells treated with DMSO were round shaped, those treated with PF-228 became more spread on laminin-332. This suggested that FAK may affect SCLC cell phenotypes relevant to disease progression and could represent a good target for therapeutic interventions in SCLC. Based on these findings and the literature, we hypothesized that FAK activation plays a key role in the invasive behaviour of SCLC and that FAK may represent a good target for therapeutic interventions. To explore this hypothesis, we proposed the following specific aims:

1/ To evaluate the role of FAK in SCLC progression in vitro by testing cell motility, invasion, proliferation, cell cycle, and apoptosis in cells where FAK is downregulated by a pharmacological method (PF-228) and by gene silencing, to confirm the selectivity of the first method. The hypothesis was that FAK inhibition by both methods will decrease cancer cell motility, invasion, and proliferation, as well as increase apoptosis.

2/ To evaluate the expression of FAK and p-FAK Y397 in SCLC and NSCLC and look for correlations with clinical characteristics The hypothesis was that FAK was a prognostic biomarker in lung cancer, especially SCLC.

The impact of such research is that understanding the role of FAK in SCLC may provide greater insight into the molecular steps leading to SCLC progression and, ultimately, may justify the development of FAK-targeted therapeutic strategies in order to reduce mortality from this aggressive cancer.

3 INCREASED EXPRESSION AND ACTIVATION OF FAK IN SMALL-CELL LUNG CANCER COMPARED TO NON-SMALL-CELL LUNG CANCER

(Published in Cancers **2019**, 11, 1526)

Frank Aboubakar Nana ^{1,2}, Delphine Hoton ³, Jérôme Ambroise ⁴, Marylène Lecocq ¹, Marie Vanderputten ¹, Yves Sibille ^{1,5}, Bart Vanaudenaerde ⁶, Charles Pilette ^{1,2}, Caroline Bouzin ⁷ and Sebahat Ocak ^{1,5,*}

¹ Pole of Pneumology, ENT, and Dermatology (PNEU), Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium;

² Division of Pneumology, Cliniques Universitaires St-Luc, UCLouvain, Brussels, Belgium;

³ Department of Pathology, Cliniques Universitaires Saint-Luc, UCLouvain, Brussels, Belgium;

⁴ Centre de Technologies Moléculaires Appliquées, IREC, UCLouvain, Brussels, Belgium;

⁵ Division of Pneumology, CHU UCL Namur (Godinne Site), UCLouvain, Yvoir, Belgium;

⁶ Lung Transplant Unit, Division of Respiratory Disease, Department of Clinical and Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium;

⁷ Imaging Platform, IREC, UCLouvain, Brussels, Belgium

* Correspondence: sebahat.ocak@uclouvain.be; Tel.: +32-2-764-9448; Fax.: +32-2-764-9440

3.1 Abstract

Introduction: Focal adhesion kinase (FAK) plays a crucial role in cancer development and progression. FAK is overexpressed and/or activated and associated with poor prognosis in various malignancies. However, in lung cancer, activated FAK expression and its prognostic value are unknown. Methods: FAK and activated FAK

(phospho-FAK Y397) expressions were analyzed by multiplex immunofluorescence staining in formalin-fixed paraffin-embedded tissues from 95 non-small-cell lung cancer (NSCLC) and 105 small-cell lung cancer (SCLC) patients, and 37 healthy donors. The FAK staining score was defined as the percentage (%) of FAK-stained tumor area multiplied by (×) FAK mean intensity and phospho-FAK staining score as the (% of phospho-FAK-stained area of low intensity × 1) + (% of phospho-FAK-stained area of medium intensity × 2) + (% of the phospho-FAK-stained area of high intensity × 3). FAK and phospho-FAK staining scores were compared between normal, NSCLC, and SCLC tissues. They were also tested for correlations with patient characteristics and clinical outcomes. Results: The median follow-up time after the first treatment was 42.5 months and 6.4 months for NSCLC and SCLC patients, respectively. FAK and phospho-FAK staining scores were significantly higher in lung cancer than in normal lung and significantly higher in SCLC compared to NSCLC tissues ($p < 0.01$). Moreover, the ratio between phospho-FAK and FAK staining scores was significantly higher in SCLC than in NSCLC tissues ($p < 0.01$). However, FAK and activated FAK expression in lung cancer did not correlate with recurrence-free and overall survival in NSCLC and SCLC patients. Conclusions: Total FAK and activated FAK expressions are significantly higher in lung cancer than in normal lung, and significantly higher in SCLC compared to NSCLC, but are not prognostic biomarkers in this study. View Full-Text

Keywords: expression; FAK; lung cancer; small-cell lung cancer; non-small-cell lung cancer; multiplex immunofluorescence staining; phospho-FAK; prognosis; targeted therapy

3.2 Introduction

Lung cancer is histologically divided into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), representing 85% and 15% of the cases, respectively [18,20]. These last years, oncogenic drivers with sensitivity to targeted therapies (e.g.: tyrosine kinase inhibitors (TKIs) targeting epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, or other oncogenic abnormalities) have been discovered in NSCLC, leading to improvements in the outcome of oncogenic-driven NSCLC patients [150]. Immunotherapy with anti-programmed death-ligand 1 (PD-L1) immune checkpoint inhibitors (ICIs) has also significantly improved the 5-year overall survival (OS) of metastatic NSCLC patients without oncogenic drivers from 6% to 15% [29,458]. Clinically, SCLC is the most aggressive type of lung cancer, characterized by a high growth rate and a tendency for early metastasis, with two third of the patients diagnosed with an extensive stage (ES) disease and a 5-year OS as low as 5% [20]. Despite improved understanding of the molecular steps leading to SCLC development and progression these last years, there is still no effective targeted therapies in SCLC, as opposed to NSCLC. After four decades, the only modest improvement in OS of patients suffering from ES-SCLC has recently

been shown in a trial combining atezolizumab, an anti-PD-L1 immune checkpoint inhibitor, with carboplatin and etoposide, chemotherapy agents [62].

Focal Adhesion Kinase (FAK) is a 125 kDa cytosolic non-receptor tyrosine kinase widely expressed in various cell types and tissues. It is localized to focal adhesions or contact points between actin cytoskeleton and extracellular matrix. Once activated by integrins, G protein-coupled receptor ligands, or growth factors and neuromediators, FAK is autophosphorylated at tyrosine 397 (Y397), then binds and activates downstream proteins such as Src, p130CAS, paxillin, and PI3KR2 [317,319,459], finally leading to cell adhesion, migration, invasion, survival, proliferation, angiogenesis, immune suppression, and regulation of DNA damage repair [317-320]. Because of these roles and its overexpression in many cancers, with correlation to poor prognosis in some of them [197,249,259,260,263,265-267,269-271,273], FAK is believed to play a role in cancer development and progression. Small-molecule inhibitors targeting FAK kinase domain (e.g.: PF-573,228) have therefore been developed as potential anti-cancer targeted therapies. They decreased FAK phosphorylation at Y397 and led to antitumoral effects in various cancer types, including NSCLC and SCLC [223,281,460,461]. In preclinical and clinical studies, they induced cancer regression or stability in several cancers, including NSCLC [208,209,211,373,461].

FAK gene copy number gain has previously been reported in 50% of 46 SCLC tissues analysed by array comparative genomic hybridization and validated by fluorescent in situ hybridization and quantitative real-time polymerase chain reaction [199]. FAK activation has also been observed in SCLC cell lines and inhibition of FAK phosphorylation at Y397 with PF-573,228 decreased cell proliferation, survival, migration, and invasion in SCLC cell lines [460]. These results suggested that FAK is important in SCLC biology and that targeting its kinase domain may have a therapeutic potential in SCLC patients. Moreover, total FAK expression has been evaluated by immunohistochemistry (IHC) in tissue microarrays (TMAs) including SCLC tissues from 85 patients, revealing an expression of FAK in 92% of the tumors, scored low in only 13%, while moderate in 20% and strong in 59% of the samples [195]. However, no correlation was found between total FAK expression and recurrence-free survival (RFS) or OS in these SCLC patients [195]. Nevertheless, total FAK expression does not necessarily indicate an activated FAK pathway, as opposed to phospho-FAK expression.

Because there is a lack of data evaluating the expression of phospho-FAK in human lung cancer tissues as opposed to total FAK expression [192,195,197], we aimed to evaluate the expression of phospho-FAK (Y397) in SCLC and NSCLC tissues, and correlate the data to patients' prognosis.

3.3 Materials and methods

3.3.1 Patients and tissues samples

Formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients with lung cancer and healthy donors were obtained from the tumor registry of Cliniques Universitaires St-Luc, CHU UCL Namur (Godinne Site), and Katholieke Universiteit Leuven. Lung cancer tissues were collected between January 2011 and February 2016 from 95 NSCLC and 105 SCLC patients at the time of diagnosis, before any medical treatment. Normal lung samples, used as controls, were collected from 37 healthy donors between February 2016 and March 2019. All tumor sections were reviewed by an experienced lung cancer pathologist (D.H.) and only tumor sections with representative areas of tumor and adjacent lung parenchyma were included in the study. Sixty-seven of the NSCLC tissues were represented in TMAs (prepared in accordance with reported methods) [462,463], while none of the SCLC tissues were because they were all transbronchial or transthoracic biopsies, with no surgical specimens, as opposed to NSCLC tissues.

Treatment was administered on an individual basis according to disease stage and patient performance status as per standard of care. All patients were followed with chart review until death or until data analysis of the manuscript. Clinical data were obtained from tumor registry and hospital charts. Histological classification of tumours was based on the World Health Organization criteria [464]. All tumours were staged according to the 7th lung cancer TNM pathological classification and staging system of the International Union Against Cancer (UICC) [27]. Patient characteristics are summarized in Tables 1A and 1B. This study was approved by institutional ethical review board (CHU UCL Namur (Godinne)) at each medical center (number of approval: 115/2014). The normal lung samples were obtained from unused lungs of donors and collected according to existing Belgian law and approved by the hospital's ethical committee (S59648, S61653).

3.3.2 Multiplex immunofluorescence immunohistochemistry (mIF-IHC)

FFPE tissue blocks were sectioned at 5 μ m. After deparaffinization in toluene and methanol, endogenous peroxidases were inhibited 15 min. in Bloxall (Vector Laboratories, Peterborough, United Kingdom) followed by 30 min. in 0.3% hydrogen peroxide. Sections were then submitted to microwave antigen retrieval in 10 mM citrate pH 6.0 buffer containing 0.1% triton and to blocking of aspecific antigen binding sites (Tris buffered saline (TBS) containing 5% normal goat serum and 0.1% Tween 20). The first primary antibody was incubated in TBS containing 1% normal goat serum and 0.1% Tween 20 and detected by corresponding horseradish peroxidase (HRP)-conjugated polymer secondary antibodies for 40 min. at room

temperature (RT). HRP was then visualized by tyramide signal amplification (TSA) using AlexaFluor-conjugated tyramides (Thermo Fisher Scientific, Paisley, United Kingdom). After a new citrate buffer incubation step, the same protocol was applied with other primary antibodies and different AlexaFluor or fluorescein-conjugated tyramides. In this study, two sequential incubations with phospho-FAK Y397 rabbit antibody (0.5 µg/ml, 1 hour at RT; Thermo Fisher Scientific) and total FAK mouse antibody (5 µg/ml, overnight at 4°C; Thermo Fisher Scientific) were performed. Total FAK and phospho-FAK Y397 were respectively revealed with TSA-conjugated fluorophores, AF647 and AF594 (1:150 dilution in 0.1M Borate pH 7.8 buffer, 10 min. at RT; Thermo Fisher Scientific). Finally, nuclei were counterstained with Hoechst 33342 (Thermo Fisher Scientific) diluted in TBS containing 10% BSA and 0.1% Tween 20, washed in TBS containing 0.1% Tween 20, and mounted with Dako fluorescence mounting medium (Dako, Glostrup, Denmark). Negative controls were established by adding nonspecific isotype controls as primary antibodies. Slides were stored at -20°C until multispectral image acquisition.

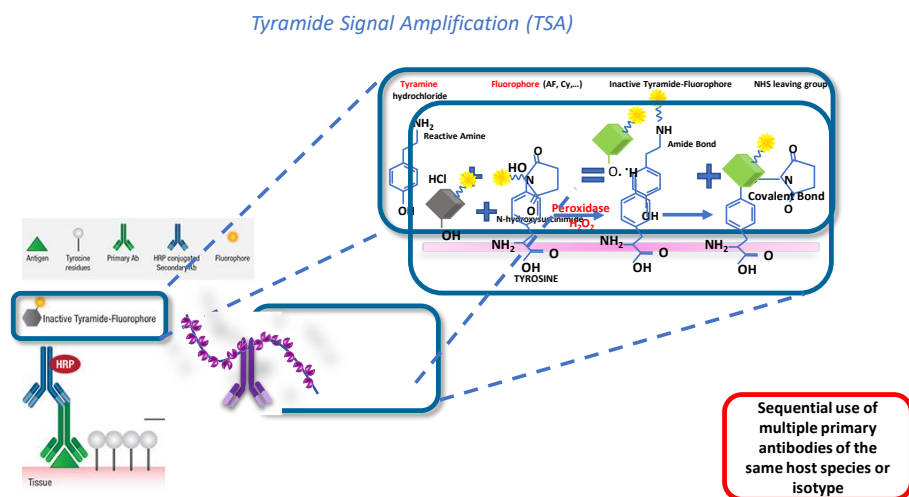


Figure 28: Tyramide signal amplification and multiplex immunostaining principle. Peroxidases, coupled to secondary antibodies, catalyze the deposition of several tyramide-fluorophore molecules at the site of the antigen. The covalent nature of tyramide-tyrosine bound allows for heat-mediated removal (stripping) of primary/secondary antibody pairs bound to the antigen, while preserving the antigen-associated fluorescence signal. This allows the sequential use of multiple primary antibodies of the same host species or isotype without the concern for

crosstalk, thereby greatly enabling multiplexing potential on the same slide sample.

After image acquisition, coverslips were removed by immersion of slides into water overnight at RT. Sections were incubated with 5% human serum before adding anti-pan cytokeratin CKAE1-AE3 antibody (Tu sais pas enlever l'ombre du cadre rouge?1:200, 1 hour at RT; Dako) followed by HRP-conjugated polymer secondary antibody for tumor detection. Peroxidase activity was revealed through 5 min. incubation with diaminobenzidine (DAB) substrate (IM2394; Immunotech, Marseille, France). Slides were finally counterstained for 3 min. with hematoxylin (Dako).

3.3.3 Stained slides imaging

Multiplex immunofluorescence immunostained slides were digitalized in fluorescence using a Panoramic 250 FlashIII scanner (3DHistech, Budapest, Hungary) at 20x magnification using the following filter cubes: DAPI1 (ex: 377/50 nm - em: 477/60 nm), SpRed (ex: 586/20 nm - em: 628/32 nm), and Cy5 (ex: 328/40 nm - em: 692/40 nm). After pan- CKAE1-AE3 staining, slides were re-scanned in brightfield at the same magnification (**Fig.29**).

3.3.4 Quantitative evaluation of immunostaining

FAK and phospho-FAK stainings were quantified on multiplex-stained paraffin sections (TMA or not) with software applications (APP) using the image analysis tool Oncotopix version 2017.2 (Visiopharm, Hørsholm, Denmark).

Fluorescent (p-FAK-FAK) and brightfield (pan-CK-hematoxylin) scans were first merged using the Tissuealign add-on from Visiopharm in order to be able to delineate tumor compartments (tumor clusters/stroma) on the brightfield scan and to subsequently detect and quantify FAK and p-FAK in tumor cells on the fluorescent scan.

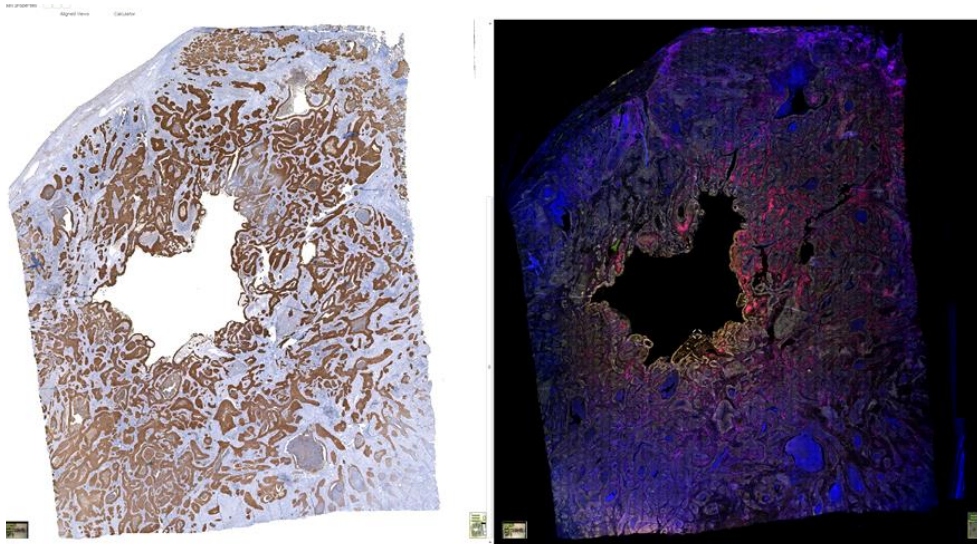


Figure 29: Fluorescent (p-FAK-FAK) and brightfield (pan-CK-hematoxylin) sequential image acquisition.

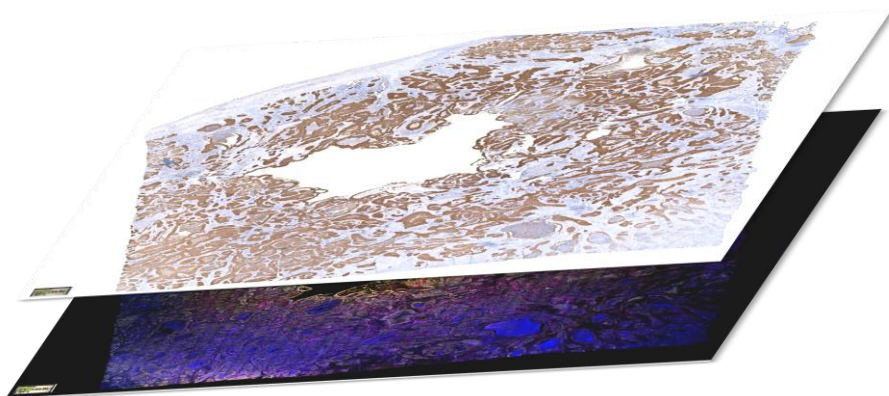


Figure 30: Fluorescent and brightfield images alignment.

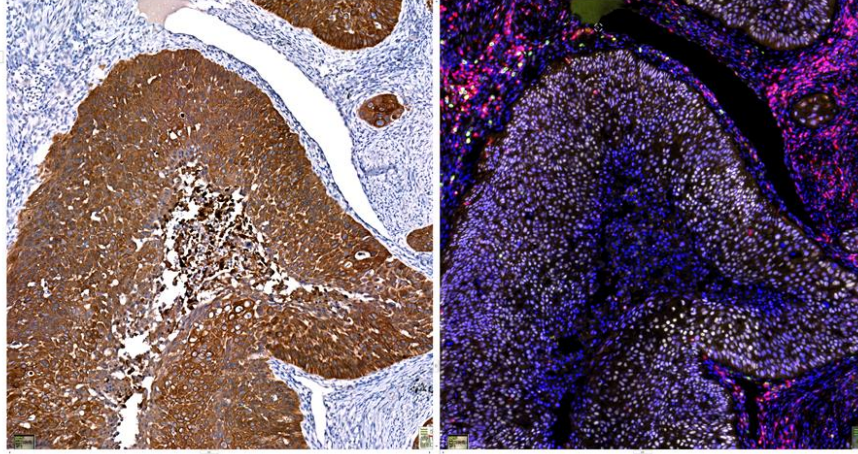


Figure 31: Fluorescent (p-FAK-FAK) and brightfield (pan-CK-hematoxylin) scans merging and alignment.

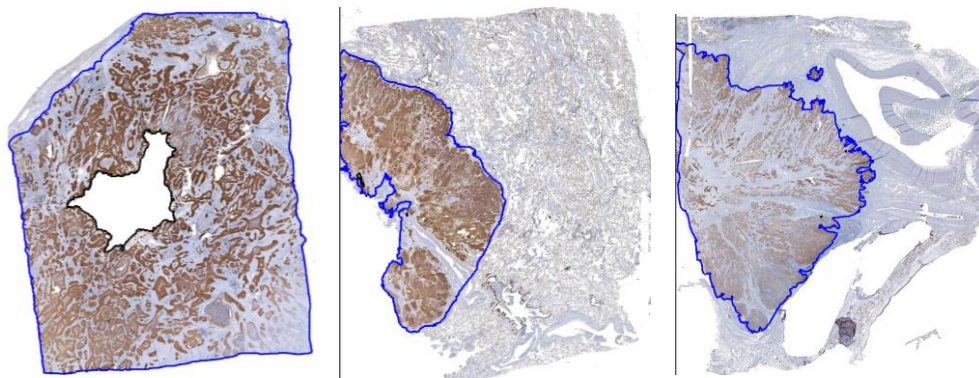


Figure 32: Tumor delineation: detection based on pan-CK detection.

Using a first APP, tumor was delineated based on CKAE1-AE3 staining at a low digital magnification (3x) using a thresholding classification method based on the HDAB-DAB feature of the software and post-processing steps designed to fill the detected area (**Fig.31 and 32**) and to outline it within a region of interest (ROI). For TMA sections, each TMA plug was outlined with a different ROI (Fig. 1). Within the delineated tumor, CKAE1-AE3-stained tumor clusters were delineated in a second APP at a low digital magnification (5x) using a thresholding classification method based on the HDAB-DAB feature of the software (**Fig.31 and 32**). Large empty spaces (alveolae, vessels, and damaged tissues) were discarded. Delineated scans were then duplicated to proceed in parallel to the detection and quantification of FAK and phospho-FAK at high magnification (20x).

These two first delineation operations were visually checked and manually corrected if required. Glands presenting aspecific red fluorescence were excluded from the analysis.

FAK-stained areas were detected with a thresholding classification method on the Alexa fluor 647 staining. Mean fluorescence intensity of the pixels within the tumor cluster ROI was also calculated. FAK expression results were reported using a FAK staining score, corresponding to the percentage (%) of FAK-stained tumor area multiplied by (*) FAK mean intensity. Phospho-FAK stained areas were detected using three thresholds of intensity of the AlexaFluor594 to highlight the differences in staining intensity, much lower than total FAK and therefore requiring a different method of detection. Phospho-FAK expression results were reported using a phospho-FAK staining score, corresponding to (% of phospho-FAK-stained tumor area of low intensity * 1) + (% of phospho-FAK-stained tumor area of medium intensity * 2) + (% of phospho-FAK-stained tumor area of high intensity * 3). Similar calculations were used to evaluate FAK and phospho-FAK staining scores in the nuclei (detected with a thresholding classification method based on the Hoechst nuclear counterstaining).

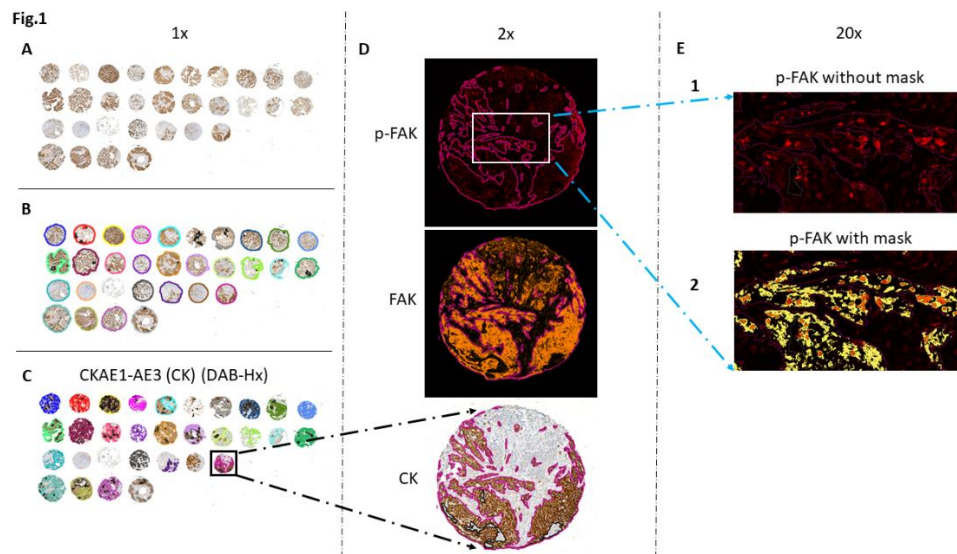


Figure 33: Illustration of FAK and phospho-FAK staining quantification on a tissue microarray section of non-small-cell lung cancer (NSCLC) stained by multiplex immunofluorescence (IF) immunohistochemistry (IHC). (A) TMA sections were sequentially stained by mIF with an antibody against phospho-FAK (red signal) and FAK (orange signal), followed by Hoechst nuclear marker (blue signal). After whole slide fluorescence image acquisitions, IHC was performed with a tumor marker using an

antibody against pan-cytokeratin CKAE1-AE3 (CK, brown signal) on the same slide and digitalized with a slide scanner. (B) Each TMA plug was then automatically delineated via the image analysis tool Oncotopix version 2017.2 (Visiopharm). (C) CK-positive tumor regions were semi-automatically delineated from CK-negative stroma. (D) These tumor regions, detected on the brightfield scan, were transposed to the aligned fluorescent scan with Visiopharm Tissue Align module. (E) FAK and phospho-FAK stained areas were finally detected and quantified as illustrated for phospho-FAK in Fig. D.2., with staining detection according to three thresholds of intensity (low, yellow; medium, orange; high, red), while Fig. D.1. shows phospho-FAK staining without the mask. *Original magnification: A, B, C: 1x; D: 2x; E: 20x.*

3.3.5 Western blot

Thirty frozen NSCLC, 10 frozen SCLC, and nine frozen normal lung tissues were lysed with 250 µl of RIPA buffer with anti-protease and anti-phosphatase agents (Roche Diagnostics, Mannheim, Germany). Equal amounts of lysate were separated by 12% SDS-PAGE and electrotransferred onto a nitrocellulose membrane. After blocking 1h with 5% W/V BSA (Sigma, Saint-Louis, MO) in TBS with 0.1% Tween 20 (Sigma), the membrane was incubated overnight at 4°C with phospho-FAK Y397 rabbit antibody (1/1,000 Cell Signaling Technology, Danvers, MA) or total FAK mouse antibody (1/250, Santa Cruz Biotechnology, Dallas, TX) and GAPDH rabbit antibody (1/5,000, Sigma). Secondary antibodies consisted of HRP-conjugated goat anti-rabbit IgG (Cell Signaling Technology) or HRP-conjugated goat anti-mouse IgG (Sigma). Immunoreactivity bands were developed using chemiluminescence (Amersham ECL, GE Healthcare, Little Chalfont, Buckinghamshire, UK) and detected with a chemidoc XRS apparatus (Bio-rad, Hercule, CA) and quantified using the Quantity One software (Bio-rad).

3.3.6 Statistical analysis

RFS and OS were computed for all patients as the time between first treatment (i.e.: surgery, chemoradiation, or first line chemotherapy) and the first relapse or death. Patients were right censored at the time of their last date of physical examination when they were still alive and without relapse at the time of analysis. Univariate and multivariate hazard ratios were computed on RFS using univariate and multivariate Cox proportional hazard regression models. P-values were obtained using linear models and adjusted for multiple testing using the Bonferroni method. All data were analysed using R.3.4.0. A p-value (P) < 0.05 was considered to be statistically significant.

3.4 Results

3.4.1 Patient Characteristics

A total of 95 patients diagnosed with NSCLC were included in the study based on the availability of archival pathology specimens. NSCLC patient characteristics are described in **Table 4**. Of the 95 patients, 26 (27.4 %) were women and 69 (72.6%) were men. Only four (4.2 %) patients had never smoked and one (1.1%) had an unknown smoking history, while all the others were current (n=33, 34.7%) or ex-smokers (n=57, 60.0 %) with a median pack-year history of 40 (range: 2-107). The median age at diagnosis was 66 years (range: 30 -85) with 21 (22.1 %) patients older than 75 years. The disease was stage I for 45 (47.4 %), II for 23 (24.2%), III for 19 (20.0%), and IV for eight (8.4 %) patients. All patients received medical treatment according to disease stage and performance status as per standard of care therapy. Recurrence free survival (RFS) was assessed according to Response Evaluation Criteria in Solid Tumors guidelines and was available for all patients. The median time of follow-up after first treatment was 42.5 months (range: 1.3 - 92.4). At two and five years, the RFS was 76.6 % (95% CI: 68.3 – 85.9 %) and 67.5% (95% CI: 58.2 - 78.3 %), respectively. At two and five years, OS was 80.9 % (95% CI: 73.3 - 89.2 %) and 66.2% (95% CI: 56.9 - 77.0 %), respectively.

A total of 105 patients diagnosed with SCLC were included in the study based on the availability of archival pathology specimens. SCLC patient characteristics are described in **Table 5**. Of the 105 patients, 38 (36.2 %) were women and 67 (63.8.6%) were men. Only one (1.0 %) patient had never smoked and 10 (9.5%) had an unknown smoking history, while all the others were current or ex-smokers with a median pack-year history of 43 (range: 1-170). The median age at diagnosis was 66 years (range: 43 -89) with 27 (25.7 %) patients older than 75 years. The disease stage was extensive for 69 (65.7 %), limited for 21 (20.0%), and unknown for 15 (14.3%) patients. The median time of follow-up after first treatment was 6.4 months (range: 0.1 - 79.0). At two and five years, the RFS was 13.1 % (95% CI: 7.8 - 22.0 %) and 7.6% (95% CI: 2.7 - 15.5 %), respectively. At two and five years, the OS was 20.2 % (95% CI: 13.7 – 29.7 %) and 7.0% (95% CI: 3.3 - 15.0 %), respectively.

A total of 37 healthy donors provided normal lung tissues. Their clinical characteristics are described in **Table 6**. Of the 37 patients, three (8.1 %) were women and 34 (91.9%) were men. Eight of them (21.6 %) had never smoked, 10 (27.0%) were current or ex-smokers, and 19 (51.4%) had an unknown smoking history. The median age at diagnosis was 59.5 years (range: 19 -79)

Variable	NSCLC patients (n=95)
Histological subtype	
Squamous	34 (35.8)
Non squamous	61 (64.2)
Stage - n (%)	
I	45 (47.4)
II	23 (24.2)
III	19 (20.0)
IV	8 (8.4)
Gender - n (%)	
Female	26 (27.4)
Male	69 (72.6)
Age at diagnostic >75 y/o - n (%)	
No	74 (77.9)
Yes	21 (22.1)
Smoking history - n (%)	
Unknown	1 (1.1)
Never	4 (4.2)
Ex	57 (60.0)
Current	33 (34.7)
Pack per year	
Median	40
Range	2 - 107

Table 4: Clinical and pathological characteristics of NSCLC patients.

Variable	SCLC patients (n=105)
Stage - n (%)	
ED	69 (65.7)
LD	21 (20.0)
Unknown	15 (14.3)
Gender - n (%)	
Female	38 (36.2)
Male	67 (63.8)
Age at diagnostic >75 y/o - n (%)	
No	78 (74.3)
Yes	27 (25.7)
Smoking history - n (%)	
Unknown	10 (9.5)
Never	1 (1.0)
Ex	32 (30.5)
Current	62 (59.0)
Pack per year	
Median	43
Range	1 - 170

Table 5: Clinical and pathological characteristics of SCLC patients.

Variable	Healthy donors (n=37)
Gender - n (%)	
Female	3 (8.1)
Male	34 (91.9)
Age >75 y/o - n (%)	
No	36 (97.3)
Yes	1 (2.7)
Agee (year)	
Median	59.5
Range	19-79
Smoking history - n (%)	
Unknown	19 (51.4)
Never	8 (21.6)
Yes	10 (27.0)

Table 6: Clinical and pathological characteristics of healthy donors.

Abbreviations: ED, extensive-stage disease; LD, limited-stage disease; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer, y/o: years old.

3.4.2 FAK expression and activity are higher in SCLC than NSCLC and normal lung

FAK and phospho-FAK (Y397) staining pattern in NSCLC whole slide samples was homogenous, with either all the cells staining for FAK or phospho-FAK or none at all, although the staining intensity was clearly different between samples (**Fig. 34 A-B-C-D**). Based on this observation, we concluded that FAK staining quantification could also be performed on NSCLC samples organized in TMAs (available for NSCLC but not for SCLC).

FAK expression was mainly cytoplasmic while phospho-FAK (Y397) staining was mainly nuclear, both in NSCLC (**Fig. 34 A-B-C-D**) and SCLC (**Fig. 34 E**). FAK staining was found in tumor and normal broncho-epithelial cells, as well as in immune and endothelial cells from the tumor microenvironment. Phospho-FAK was mainly expressed in tumor, endothelial, and some immune cells but not in normal broncho-

epithelial cells (**Fig. 2A-2B-2C-2D-2G**). Of note, peritumoral normal lung and tumor microenvironment were observed only in NSCLC samples because SCLC biopsies were small and almost exclusively consisting of tumor cells.

FAK expression was significantly higher in SCLC compared with NSCLC and normal lung tissues as assessed by mean FAK staining scores (11863 ± 5798 vs 8727 ± 4501 vs 418 ± 468 , respectively) ($P < 0.01$) (**Fig. 35 A**). FAK activity, represented by phospho-FAK (Y397) expression, was predominantly found in tumor cells whereas its expression was low in normal lung alveoli and interstitial tissue (**Fig. 34 A-B-C-D**). Phospho-FAK (Y397) expression was significantly increased in SCLC compared with NSCLC and normal tissues as assessed by mean phospho-FAK staining scores (146 ± 50 vs 67 ± 32 vs 17 ± 11 , respectively) ($P < 0.01$) (**Fig. 35 B**). Interestingly, the proportion of activated FAK compared with total FAK expression was significantly increased in SCLC as compared with NSCLC, as assessed by the ratio between mean phospho-FAK staining score and mean FAK staining score (0.025 ± 0.063 vs 0.011 ± 0.014) ($P < 0.01$) (**Fig. 35 C**).

In a second step, we specifically evaluated FAK and phosphor-FAK nuclear staining. We found that mean nuclear FAK staining scores were significantly increased in lung cancer as compared to normal lung tissues, but without significant difference between NSCLC and SCLC (146.5 ± 61.4 vs 130.4 ± 39.4 vs 55.2 ± 19.5 , respectively) ($P < 0.01$ only for comparison between normal and lung cancer) (**Fig. 36 A**), while mean nuclear phospho-FAK staining scores were significantly increased in SCLC as compared to NSCLC and normal lung samples (91 ± 47 vs 37 ± 11 vs 25 ± 12 , respectively) ($P < 0.01$) (**Fig. 36 B**).

In order to validate the observations made by mIF-IHC, we also evaluated FAK and phospho-FAK expression by WB in 10 SCLC, 30 NSCLC, and nine normal lung tissue lysates. This technique confirmed a significant increase in SCLC, compared with NSCLC and normal lung tissues, of FAK expression (0.177 ± 0.169 vs 0.052 ± 0.066 vs 0.013 ± 0.024 , respectively) ($P = 0.04$) (**Fig. 37 A and C**) and phospho-FAK expression (0.727 ± 0.448 vs 0.021 ± 0.053 vs 0.056 ± 0.09) ($P < 0.001$) (**Fig. 37 B and C**).

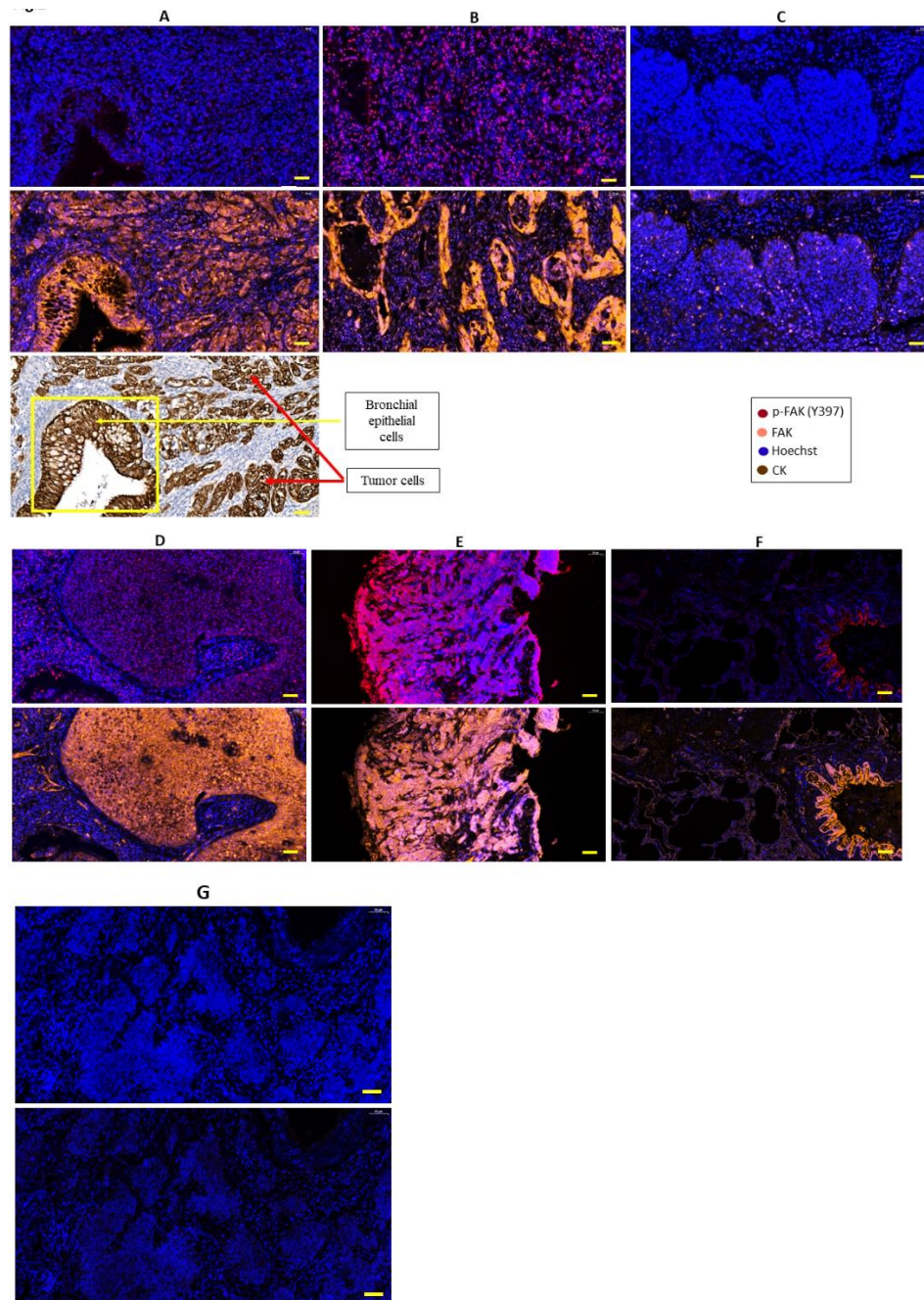


Figure 34: Illustrations of FAK and phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence (IF) immunohistochemistry (IHC) in lung cancer and normal lung tissues. (A) Lung adenocarcinoma with

absence of phospho-FAK expression but homogenous cytoplasmic FAK staining (orange) in tumor core, adjacent non-tumoral bronchi, and some stromal cells (including vessels and lymphoid structures). (B) Lung adenocarcinoma with nuclear phospho-FAK staining (red) and homogenous cytoplasmic FAK staining (orange). (C) Lung squamous carcinoma with absence of phospho-FAK expression but weak cytoplasmic FAK staining. (D) Lung squamous carcinoma with nuclear phospho-FAK staining (red) and homogenous cytoplasmic FAK staining (orange). (E) Small-cell lung cancer with nuclear phospho-FAK staining (red) and cytoplasmic FAK staining (orange). (F) Normal lung with cytoplasmic FAK staining in bronchi and some stromal cells (including vessels and lymphoid structures). (G) Lung squamous carcinoma used as negative control, showing the absence of phospho-FAK and FAK staining. Original magnification: 20x; scale bar: 50 μ m.

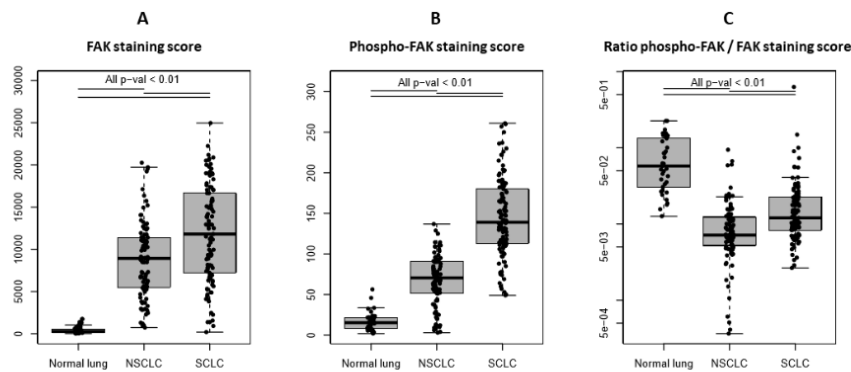


Figure 35: Quantification of FAK and phospho-FAK (Y397) expression evaluated by multiplex immunofluorescenceimmunohistochemistry in 37 normal lung, 95 non-small-cell lung cancer (NSCLC), and 105 small-cell lung cancer (SCLC) tissues: (A) FAK staining score: percentage (%) of FAK-stained tumor area multiplied by (*) FAK mean intensity, (B) phospho-FAK (Y397) staining score: (% of phospho-FAK-stained tumor area of low intensity * 1) + (% of phospho-FAK-stained tumor area of medium intensity * 2) + (% of phospho-FAK-stained tumor area of high intensity * 3), and (C) ratio between phospho-FAK and FAK staining scores. Each dot represents one sample. Data presented as the mean $\pm$ S.D. P-values were obtained using linear models and adjusted for multiple testing using the Bonferroni method.

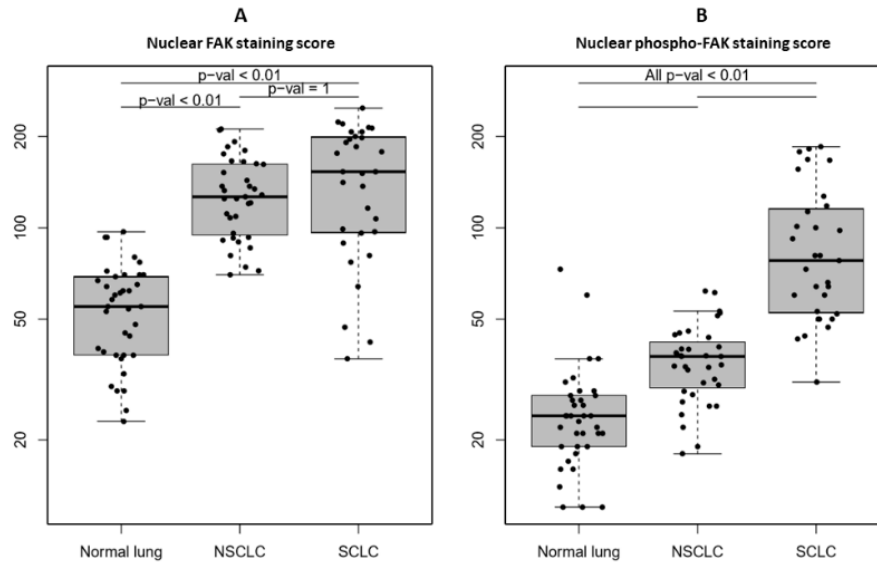


Figure 36: Quantification of nuclear FAK and nuclear phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence immunohistochemistry in 37 normal lung, 95 non-small-cell lung cancer (NSCLC), and 105 small-cell lung cancer (SCLC) tissues: (A) nuclear FAK staining score: percentage (%) of FAK-stained nucleus area multiplied by (*) nuclear FAK mean intensity, (B) nuclear phospho-FAK (Y397) staining score: (% of phospho-FAK-stained nucleus area of low intensity * 1) + (% of phospho-FAK-stained nucleus area of medium intensity * 2) + (% of phospho-FAK-stained nucleus area of high intensity * 3). Each dot represents one sample. Data presented as the mean $\pm$ S.D. P-values were obtained using linear models and adjusted for multiple testing using the Bonferroni method.

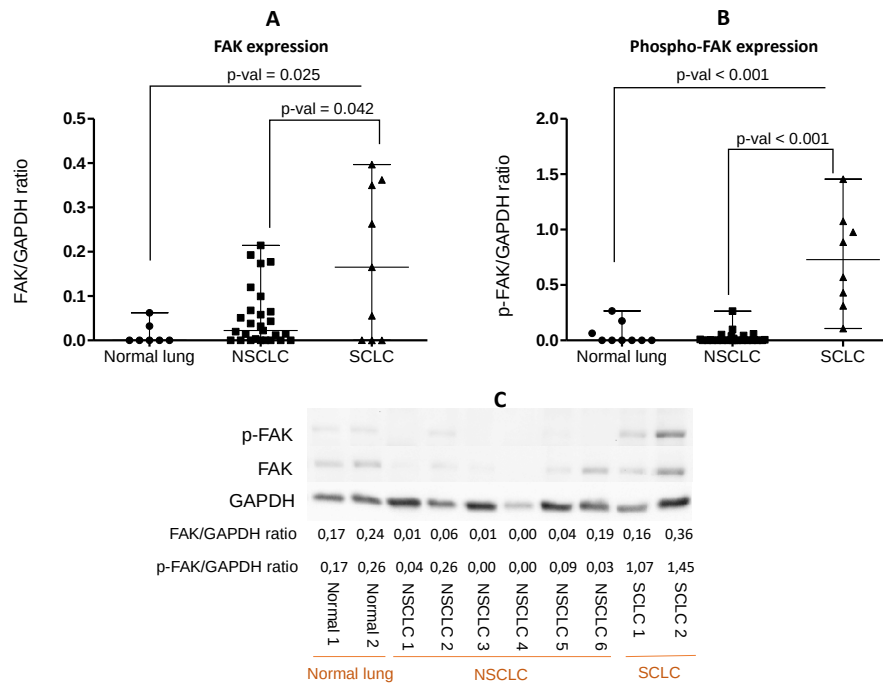


Figure 37: Quantification of (A) FAK and (B) phospho-FAK expression evaluated by Western blot (WB), with normalization to GAPDH expression, in nine normal lung, 30 non-small-cell lung cancer (NSCLC), and 10 small-cell lung cancer (SCLC) tissue lysates. Each dot represents one sample. Data presented as the mean $\pm$ S.D. Significance determined by Kruskal-Wallis test. (C) Illustration of a representative WB of FAK and phospho-FAK (Y397) expression in normal lung, NSCLC, and SCLC tissue lysates. All the WB are represented in Supplementary Fig. S1.

3.4.3 FAK expression and activity do not correlate with patient characteristics or survival

The availability of clinical data for each sample from the NSCLC (n=95) and the SCLC (n=105) patients enabled to assess the impact of FAK expression and activity on survival outcomes. Univariate analysis of staining scores treated as continuous variables showed no significant correlation of FAK and phospho-FAK expression with RFS and OS in NSCLC (**Table 7 A**) and SCLC (**Table 7 B**) patients.

In a multivariate analysis including disease stage, age at diagnosis, smoking history, and histological subtype, no significant association was found between phospho-FAK staining score and RFS or OS in NSCLC patients (**Table 8 A**). Similarly, in a

multivariate analysis including disease stage, age at diagnosis, and smoking history, the ratio between phospho-FAK staining score and FAK staining score was not significantly associated with RFS or OS in SCLC patients (**Table 8 B**). As expected, disease stage was the most significant independent predictor of RFS and OS both in NSCLC and SCLC.

Variable	Recurrence-free survival		Overall survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value
A				
FAK staining score	1.00 (1.00 - 1.00)	0.92	1.00 (1.00 - 1.00)	0.99
Phospho-FAK staining score	0.99 (0.98 - 1.00)	0.1	0.99 (0.98 - 1.00)	0.21
Ratio phospho-FAK / FAK staining scores	0.86 (0.60 - 1.23)	0.33	0.89 (0.64 - 1.21)	0.4
B				
FAK staining score	1.00 (1.00 - 1.00)	0.76	1.00 (1.00 - 1.00)	0.66
Phospho-FAK staining score	1.00 (1.00 - 1.01)	0.5	1.00 (0.99 - 1.00)	0.8
100 x ratio phospho-FAK / FAK staining scores	0.96 (0.90 - 1.02)	0.13	1.00 (0.97 - 1.03)	0.75

Table 7: Correlation of FAK and phospho-FAK expression with recurrence-free survival and overall survival in (A) NSCLC and (B) SCLC patients in a univariate analysis.

Abbreviations: CI, confidence interval; FAK, focal adhesion kinase; HR, hazard ratio.

A				
Variable	Recurrence-Free survival		Overall survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Stage (ref: I)				
II	1.06 (0.35 - 3.20)	0.92	0.63 (0.19 - 2.04)	0.44
III	1.92 (0.72 - 5.16)	0.19	2.76 (1.11 - 6.83)	0.03
IV	5.64 (1.79 - 17.7)	<0.01	3.21 (1.06 - 9.66)	0.04

Age at diagnostic (ref: <75 y/o)				
> 75 y/o	1.19 (0.49 -- 3.07)	0.72	0.75 (0.28 - 2.03)	0.57
Smoking history (ref: ex and never)				
Current	0.82 (0.35 - 1.89)	0.64	0.88 (0.39 - 1.96)	0.75
Histology (ref: ADC)				
SCC	0.66 (0.26 - 1.69)	0.39	1.59 (0.70 - 3.62)	0.27
Phospho-FAK staining score	0.99 (0.98 - 1.00)	0.15	0.99 (0.98 - 1.01)	0.30

B				
	Recurrence-Free survival		Overall survival	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Stage (ref: LD)				
ED	3.75 (2.08 - 6.78)	<0.01	3.47 (1.91 - 6.32)	<0.01
Age at diagnostic (ref: <75 y/o)				
> 75 y/o	1.56 (0.93 - 2.63)	0.09	1.42 (0.88 - 2.29)	0.15
Smoking history (ref: ex and never)				
Current	1.23 (0.78 - 1.93)	0.37	1.11 (0.73 - 1.70)	0.62
100 x ratio phospho-FAK / FAK staining scores	0.95 (0.89 - 1.02)	0.14	1.00 (0.97 - 1.03)	0.87

Table 8: . Multivariate Cox proportional regression analysis for the association with recurrence-free survival and overall survival of (A) phospho-FAK staining score in NSCLC (n=95) and (B) the ratio between phospho-FAK and FAK staining scores in SCLC patients (n=105)

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; ED, extensive-stage disease; LD, limited-stage disease; FAK, focal adhesion kinase; HR, hazard ratio; SCC, squamous cell carcinoma; y/o: years old.

3.5 Discussion

In this study, we showed by mIF-IHC that FAK and phospho-FAK are both significantly overexpressed in lung cancer as compared to normal lung tissues.

Interestingly, we also showed that, among lung cancers, FAK and phospho-FAK expression, as well as the ratio between phospho-FAK and FAK expression are significantly higher in SCLC compared to NSCLC. Moreover, we validated these observations by WB of NSCLC, SCLC, and normal lung tissue lysates. However, we did not find any correlation between FAK and activated FAK expression in lung cancer and RFS or OS in NSCLC and SCLC patients.

The overexpression and activation of FAK that we observed in lung cancer tissues from treatment-naïve patients, as compared to normal lung tissues, is compatible with the well-known role of FAK in cancer initiation and progression. Indeed, FAK is known to promote cell proliferation, survival, migration, invasion, angiogenesis, and immune suppression in several cancers, including lung cancer [278,317,320,363,373,374,460].

As in our study, FAK overexpression has previously been reported in many cancers [198], including NSCLC and SCLC [191-197]. Moreover, FAK overexpression has been associated with poor survival in various cancers [246,259,263,270,272,273]. In NSCLC, however, discordant results have been reported. In two studies including 381 [196] and 249 [193] patients with stage I-III NSCLC, FAK overexpression evaluated by IHC has been correlated with poor OS. Additionally, FAK overexpression evaluated by IHC has been correlated with increased lymph node metastasis, more advanced disease stages, and poor prognosis in a study of 153 patients with stage I- III NSCLC [197]. Nevertheless, similarly to our study, the prognostic value of FAK overexpression has not been found in a cohort of 103 patients with stage I NSCLC [192]. In SCLC, FAK expression has not been associated either with RFS and OS [195].

Unlike total FAK expression, phospho-FAK (Y397) expression represents the activation status of FAK [203,320,456] and is therefore expected to be a more relevant biomarker. Aggregation of FAK with integrins and cytoskeletal proteins in focal adhesion contacts is the best described mechanism leading to FAK activation through phosphorylation of Y397. But it is also well known that FAK can be activated by extracellular growth factors, including those released by lung cancer, such as bombesin, gastrin-related peptide (GRP), HGF, VEGF, TGF- β , HGF, and FGF [94,95,286-292]. This relationship between FAK and growth factors and neuroendocrine mediators could underline the preferential activation of FAK observed in this study in SCLC tissues, as compared to NSCLC. Increased FAK activity has already been reported in various cancer cell lines, with demonstrated antitumoral effects of FAK TKI in cancer cells where FAK was activated [206,211,223,278,465-467], including NSCLC and SCLC [281,374,460,461]. In a recent study, FAK inhibition with PF-573,228, a small-molecule TKI, decreased proliferation, survival, migration, and invasion in SCLC cell lines [199,460]. Similar

results have also been demonstrated in NSCLC cell lines, where FAK TKI decreased cell viability [281,374]. However, data related to FAK activation status in human cancer samples are scarcer. In a study including 59 patients with stage I-IV gastric carcinomas, phospho-FAK (Y397) expression evaluated by IHC was correlated with poor 5-year RFS after surgery. Interestingly, multivariate analyses showed that phospho-FAK was an independent predictor of gastric cancer recurrence rather than total FAK expression [258]. In a study of 113 patients with stage II osteosarcoma, high FAK and phospho-FAK expression by IHC was associated with poor metastasis-free and OS [268]. This result was consistent with the prognostic and predictive value of phospho-FAK overexpression reported in another study of 53 metastatic osteosarcomas [272]. In NSCLC, expression of phospho-FAK has been evaluated by WB in 44 stage I-III NSCLC frozen tissues, revealing an increased expression in NSCLC compared with normal lung tissues [468]. Furthermore, in the same study, increased phospho-FAK expression was correlated with a higher nodal involvement of cancer and a poorer RFS [468]. In another study where phospho-FAK (Y397) expression was evaluated by IHC in 145 NSCLC tissues, overexpression was found but not correlated with survival [469].

To our best knowledge, FAK activity has not previously been reported in SCLC human tissues. Our study is therefore the first report of phospho-FAK expression in SCLC. It is also the first report of FAK and phospho-FAK expression in a large cohort of both SCLC and NSCLC. Moreover, we provide the first comparison of FAK expression and activation status between NSCLC and SCLC tissues, showing that total FAK expression and FAK activity are both significantly higher in SCLC than in NSCLC, which suggests that the FAK pathway is more activated in SCLC than in NSCLC. Based on this observation, we may also hypothesize that the higher activation of FAK in SCLC than in NSCLC is responsible for the more aggressive biological and clinical behavior of SCLC, known for rapid growth, early and frequent metastasis, and the poorest OS among all lung cancer types. Finally, high FAK activity in SCLC suggest that FAK may be a good anti-cancer target in SCLC, alone or in combination with chemotherapy, immunotherapy, and/or radiotherapy

Despite the lack of prognostic value of total FAK and phospho-FAK expression in SCLC and NSCLC, a predictive value is not to be ruled out. Several FAK TKI have been tested in clinical trials including patients suffering from various advanced-stage cancers, which showed their antitumoral activity (up to 33% objective response rates) and safety [206,207,211]. However, there is still no biomarker to identify patients likely to respond to FAK TKI. Thus, our findings provide a framework for clinical trials evaluating FAK TKI to prospectively test total FAK and activated FAK expression, as well as the ratio between activated FAK and total FAK as potential predictive biomarkers of response to FAK TKI. This would be especially relevant for

SCLC patients, facing limited and disappointing therapeutic options, with the absence of effective targeted therapies.

In the meantime, it would also be interesting to prospectively correlate FAK expression and activity in FPPE human lung cancer tissues with response rates to FAK TKI of corresponding patient-derived xenograft models (immediate transfer of human cancer cells from NSCLC or SCLC patients to recipient immunodeficient mice).

The innovative mIF-IHC staining and quantification method we used provides an accurate FAK and phospho-FAK expression evaluation in lung cancer. This accuracy allowed us to specifically analyse FAK and phospho-FAK expression in the nucleus. Beside the well-known role of FAK in the cytosol downstream of integrin and growth factor receptor signaling, it has been shown that FAK also plays a functional role in the nucleus, where it can enter, bind to transcription factors, and regulate gene expression to influence tumorigenesis [203,425,470,471]. In this study, we showed for the first time that nuclear FAK and phospho-FAK expression is significantly higher in SCLC than in NSCLC and normal lung. Furthermore, this accuracy of mIF-IHC would be particularly relevant to evaluate and quantify FAK expression and activation status in tumor microenvironment where FAK has been shown to play a crucial role in antitumor immune evasion, for instance in pancreatic cancer [278,362]. Finally, the mIF-IHC method requires smaller amounts of sample than conventional IHC and is therefore valuable when limited tumor tissue is available, as it is usually the case in SCLC where surgical specimens are scarce because patients are rarely treated by surgery.

3.6 Conclusion

Analysis of 105 SCLC, 95 NSCLC, and 37 normal lung tissues revealed that FAK expression and activity are both significantly higher in SCLC compared with NSCLC and normal lung tissues, suggesting that the FAK pathway is more activated in SCLC than in NSCLC and that FAK may be a good anti-cancer target in SCLC, alone or in combination with chemotherapy, immunotherapy, and/or radiotherapy. Although our study did not find any correlation between FAK expression or activity and survival, suggesting that they are not prognostic biomarkers in lung cancer patients, the present workflow may be used to further assess FAK expression and activity as predictive biomarkers of response (theranostic biomarker) to FAK TKI in future clinical trials.

4 THERAPEUTIC POTENTIAL OF FOCAL ADHESION KINASE INHIBITION IN SMALL CELL LUNG CANCER

(Published in Molecular cancer therapeutics **2019**, 18, 17-27,)

Frank Aboubakar Nana¹, Marylène Lecocq¹, Maha Zohra Ladjemi¹, Bruno Detry¹, Sébastien Dupasquier¹, Olivier Feron², Pierre P. Massion³, Yves Sibille^{1,4}, Charles Pilette^{1,5,6}, Sebahat Ocak^{1,4}

¹Institut de Recherche Expérimentale et Clinique (IREC), Pôle de Pneumologie, ORL et Dermatologie (PNEU), Université catholique de Louvain (UCL), Brussels, Belgium; ²IREC, Pôle de Pharmacologie et Thérapeutique (FATH), UCL, Brussels, Belgium; ³Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center (VUMC), and Tennessee Valley Health Care Systems, Nashville, TN, USA; ⁴Division of Pneumology, CHU UCL Namur (Godinne Site), UCL, Yvoir, Belgium; ⁵Division of Pneumology, Cliniques Universitaires St-Luc, UCL, Brussels, Belgium; ⁶Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wavre, Belgium.

Running title

FAK inhibition in small cell lung cancer

Key words

FAK inhibition; FRNK; PF-573,228; Rac1; FAK shRNA; small cell lung cancer.

Financial support

This work was supported by grants from Fondation Mont-Godinne, Télévie (Fonds National de la Recherche Scientifique (FNRS)), and Fondation Willy and Marcy De Vooght, Belgium. Dr. Aboubakar was supported by Fonds Spécial de Recherche (FSR) and Télévie (FNRS), Belgium. Dr. Massion's effort was supported by the Veterans Administration I01CX001425, USA. Dr. Pilette was supported by the Fonds National de la Recherche Scientifique (1.R016.18) and by the WELBIO (CR-2012S-05). Dr. Ocak was supported by Secteurs des Sciences de la Santé, Université catholique de Louvain (UCLouvain), Belgium.

Conflicts of interest

The authors declare no potential conflict of interest.

Corresponding author

Sebahat Ocak

Institut de Recherche Expérimentale et Clinique (IREC) Pôle PNEU

Avenue Hippocrate, 54/B1-54.04 Tour Claude Bernard, +5

1200 Brussels

Belgium

e-mail: sebahat.ocak@uclouvain.be

4.1 Abstract

Small-cell lung cancer (SCLC) has a poor prognosis. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase regulating cell proliferation, survival, migration, and invasion, which is overexpressed and/or activated in several cancers, including SCLC. We wanted to determine whether FAK contributes to SCLC aggressive behavior. We first evaluated the effect of a FAK small-molecule inhibitor, PF-573,228, in NCI-H82, NCI-H146, NCI-H196, and NCI-H446 SCLC cell lines. PF-573,228 (0.1 to 5 μ M) inhibited FAK activity by decreasing phospho-FAK (Tyr397) expression, without modifying total FAK expression. PF-573,228 decreased proliferation, DNA synthesis, induced cell cycle arrest in G2/M phases, and increased apoptosis in all cell lines. PF-573,228 also decreased motility in adherent cell lines. To make sure that these effects were not off-target, we then used a genetic method to inhibit FAK in NCI-H82 and NCI-H446, namely stable transduction with FAK shRNA and/or FAK-related non-kinase (FRNK), a splice variant lacking the N-terminal and kinase domains. While FAK shRNA transduction decreased total and phospho-FAK (Tyr397) expression, it did not affect proliferation, DNA synthesis, or progression through cell cycle. However, restoration of FAK-targeting (FAT) domain (attached to focal adhesion complex where it inhibits pro-proliferative proteins such as Rac-1) by FRNK transduction inhibited proliferation, DNA synthesis, and induced apoptosis. Moreover, while FAK shRNA transduction increased active Rac1 level, FRNK re-expression in cells previously transduced with FAK shRNA decreased it. Therefore, FAK appears important in SCLC biology and targeting its kinase domain may have a therapeutic potential, while targeting its FAT domain should be avoided to prevent Rac1-mediated pro-tumoral activity.

4.2 Introduction

Lung cancer is the most common cancer and the leading cause of cancer-related death worldwide, with a median five-year overall survival of 15% [1]. Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) account for 85% and 15% of all lung cancers respectively [18]. SCLC is a neuroendocrine tumor and clinically the most aggressive type of lung cancer, characterized by a tendency for early dissemination and a five-year overall survival of 5% [20,21]. Unlike NSCLC, there is currently no targeted therapy validated in SCLC, which is the consequence of a poor understanding of its biology.

Focal Adhesion Kinase (FAK) is a non-receptor cytoplasmic tyrosine kinase and scaffold protein localized in focal adhesions, mediating and regulating signals initiated by integrins and G-protein-coupled-receptors. FAK plays a role in various cellular functions, including proliferation, survival, adhesion, migration, and invasion. The protein is composed of an N-terminal four-point-one, ezrin, radixin, moesin (FERM) domain, a central kinase domain, and a C-terminal domain [190]. Both the N-terminal and C-terminal domains mediate FAK interactions with other proteins critical for its kinase domain's activation and different cellular functions' regulation. FAK is maintained in an inactive state by the binding of the FERM domain to the kinase domain, which blocks access to the key autophosphorylation site tyrosine 397 (Tyr397)[472]. Engagement of integrins with the extracellular matrix or stimulation of G-protein-linked receptors following the binding of growth factors leads to signals that displace the FERM domain, resulting in Tyr397 autophosphorylation, changes in conformation of FAK and/or binding partners, binding and/or regulation of downstream effectors such as Src, MAPK, PI3K, paxillin, and Rac [320,473]. The C-terminal domain provides binding sites to proteins such as p130Cas and VEGFR3. It includes the focal adhesion targeting (FAT) sequence responsible for FAK's localization to focal adhesions, promoting its co-localization with integrins through interactions with integrin-associated proteins such as paxillin. The FAT domain also associates with several Rho GTPases, such as p190RhoGEF.

FAK is overexpressed and activated in several cancers and contributes to cancer progression and metastasis through its important role in cell proliferation, survival, adhesion, spreading, migration, and invasion [190,317,319]. A role of FAK in evasion of anti-tumor immunity, angiogenesis, epithelial-mesenchymal transition, regulation of cancer stem cells, DNA damage repair (DDR), and therapy resistance, including radioresistance, has also been described [373,374,386,474,475]. This role of FAK in cancer progression stimulated the development of various approaches to inhibit FAK. The first approaches used antisense FAK oligonucleotides, FAK siRNA or shRNA, and overexpression of FAK-Related Non-Kinase (FRNK), a naturally occurring splice variant of FAK which lacks the N-terminal and kinase domains and

inhibits FAK phosphorylation in a dominant negative fashion [474,476]. Inhibition of FAK through these approaches induced apoptosis and inhibited migration and angiogenesis. Since these approaches have limitations for clinical applications, small-molecule inhibitors targeting FAK kinase domain have then been developed [474,476]. They decreased Tyr397 phosphorylation and induced anti-tumoral effects in various cancer types in preclinical studies [219,223,233,467]. Moreover, some of them (e.g. PF-562,271, VS-6063, and VS-4718) showed promising clinical activities in early-phase clinical trials in patients with selected solid cancers, including NSCLC but not SCLC [190,208,211,476,477]. More recently, small-molecule inhibitors targeting different FAK scaffolding protein-protein interactions have been developed, such as inhibitors of FAK and VEGFR-3 interactions, and shown to induce anti-tumoral effects in preclinical studies [227].

However, FAK has been poorly studied in SCLC. We previously showed that it was amplified and overexpressed in SCLC tumors [195,199], and activated in SCLC cell lines [199]. Based on these observations, we hypothesized that FAK activation in SCLC contributes to its aggressive behaviour and that FAK may represent a therapeutic target for SCLC. In the present study, we therefore evaluated FAK activity in four SCLC cell lines and evaluated the effects of FAK inhibition by pharmacological (PF-573,228, PF-562,271, FAK Inhibitor 14) and genetic approaches (FAK shRNA and/or FRNK stable transduction) on cellular functions relevant for cancer progression.

4.3 Materials and methods

4.3.1 Cell culture

Four SCLC cell lines, NCI-H82, NCI-H146, NCI-446, and NCI-H196 (ATCC, Manassas, VA) were cultured in RPMI (1:1) containing heat-inactivated fetal calf serum (FCS) (10%), L-glutamine (2mM), penicillin (100U/ml), and streptomycin (100µg/ml) (Lonza, Verviers, Belgium) at 37°C, 5% CO₂. Tetracycline-free FCS (PAN-Biotech GmbH, Aidenbach, Germany) was used for FRNK transduction experiments. HEK 293FT (ATCC) cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with heat-inactivated FCS (10%), L-glutamine (2mM), penicillin (100U/ml), streptomycin (100µg/ml) (Lonza), and neomycin (500µg/ml) (Sigma, St. Louis, MO) at 37°C, 5% CO₂.

4.3.2 Drugs

PF-573,228 (PF-228) (Santa Cruz Biotechnology, Dallas, TX) and PF-562,271 (PF-271) (Sigma) were suspended in DMSO (Sigma), while FAK Inhibitor 14 (Inh14) (Sigma) was suspended in water to get 10mM, 3mM, and 35mM stocks, respectively. These stocks were diluted in culture medium just before experiments to get required

concentrations (0.5 to 10 μ M for PF-228, 0.05 to 3 μ M for PF-271, and 3 to 5 μ M for Inh14). In FRNK-transduced cell lines, FRNK expression was induced by doxycycline (1 to 100ng/ml) (Sigma).

4.3.3 Lentivirus construction

Total RNA was purified using TRIzol® Reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. RNA was then reverse-transcribed to complementary DNA (cDNA) using the Revertaid H minus first strand cDNA synthesis kit with random hexamer primer (Thermo-Fisher Scientific, St. Leon-Rot, Germany). The gateway cloning system (BP and LR) Clonase® II enzyme mix (Invitrogen) was used to generate doxycycline-inducible FRNK expression lentivector. To this end, AttB1 and AttB2 sequences were respectively added to FAK's N-terminal and C-terminal by polymerase chain reaction (PCR) using the KAPA Hifi PCR kit (KapaBiosystems, Wilmington, MA) according to manufacturer's instructions. The PCR product was then purified using the Macherey-Nagel PCR purification kit (Macherey-Nagel, Düren, Germany). Purified AttB-FRNK cDNA was cloned into the pJet1.2 vector using the CloneJET PCR cloning kit (Thermo-Fisher Scientific) and transformed into chemically competent E. Coli-plasmid DNA. The plasmid was isolated using Qiagen plasmid DNA mini-prep kit (Qiagen, Hilden, Germany) and sequenced in Beckman Coulter Genomics facility (Takeley, Essex, UK). BP recombination of pJet AttB-FRNK with donor vector (Addgene Plasmid #29634) was used to generate FRNK-entry vector. This last one was recombined with pCLX-pTF-R1-DEST-R2-EBR65 lentiviral vector (Addgene Plasmid #45952) by LR recombination to generate doxycycline-inducible FRNK expression lentivector (pCLX-pTF-B1-FRNK-B2-EBR65).

4.3.4 Lentivirus production and cell lines' transduction with FAK shRNA and/or FRNK

HEK293T packaging cell lines were transfected with FAK shRNA, no-target (NT) shRNA (Sigma), FRNK plasmid, or empty vector (pCLX) using ProFection® Mammalian Transfection System (Promega, Madison, WI) in tetracycline-free medium. Virus-containing supernatants were collected 48h and 72h post-transfection and immediately used to transduce NCI-H82 and NCI-H446 at a density of 1x10⁶ cells/ml for 48h in presence of polybrene (8 μ g/ml) (Sigma)[478]. Transduced cells were selected with puromycin (2 μ g/ml) (Invivogen, Toulouse, France) and/or blasticidin (10 μ g/ml) (Invivogen) for at least ten days. The selective pressure with antibiotics was removed 24h before each experiment.

4.3.5 Western blot (WB)

SCLC cell lines cultured in 12-well flat-bottom plates in 3ml culture medium were collected (1×10^6 for NCI-H82, NCI-H146, and NCI-446; 1.5×10^5 for NCI-H196) and lysed during 0.5h on ice in 150 μ l lysis buffer (62.5mM Tris-HCl [pH 6.8], 2% lauryl sulfate sodium, 10% glycerol 50mM DTT). Equal amounts of lysates were separated by 12% SDS-PAGE, electrotransferred onto a nitrocellulose membrane, and immunoprobed with antibodies against phospho-FAK (Tyr397) (1/1000, rabbit monoclonal; Cell Signaling Technology, Danvers, MA), total FAK (1/200, rabbit polyclonal; Santa Cruz Biotechnology); PARPp85 (1/1000, rabbit polyclonal; Promega, Madison, WI), phospho-Paxillin (Tyr-118) (1/1000, rabbit polyclonal; Cell Signaling Technology), total Paxillin (1/1000, monoclonal mouse; BD Biosciences, San Diego, CA), and β -Actin (1/1000, mouse monoclonal; Sigma). Secondary antibodies consisted of HRP-conjugated goat anti-rabbit IgG (1:2000; Cell signaling Technology) or HRP-conjugated sheep anti-mouse IgG (1:10000; Sigma). Immunoreactive bands were developed using chemiluminescence (Amersham ECL; GE Healthcare, Little Chalfont, Buckinghamshire, UK), detected with a Chemidoc XRS apparatus (Bio-Rad, Hercules, CA), and densitometrically quantified using Quantity One software (Bio-Rad) (results shown in Supplementary Fig.S1).

4.3.6 Cell proliferation

NCI-H82, NCI-H146, NCI-H196, and NCI-H446 were seeded in 96-well plates in antibiotic-free medium at 6×10^4 , 4.5×10^4 , and 0.5×10^3 cells per well respectively. For pharmacological experiments, PF-228 (0.5 μ M to 10 μ M), PF-271 (0.05 to 3 μ M) or Inh14 (3 to 15 μ M) was added 24h after seeding at various concentrations and cells were cultured for up to four days. Every day, WST-1 reagent (Roche, Mannheim, Germany) was added to each well and incubated during 3h. Wells' absorbance was measured spectrophotometrically at 450nm with iMark™ microplate absorbance reader (Bio-Rad). Experiments were performed in triplicates.

4.3.7 Cell cycle analysis

Cells were seeded into 12-well plates at 0.5×10^6 cells per well. After 24h, PF-228 (0.5 μ M to 5 μ M) or DMSO was added to culture medium. After 24h-treatment, cells were pulsed with bromodeoxyuridine (BrdU) (10 μ M) for 0.5h, centrifugated, pelleted, and fixed with ice-cold ethanol (70%). DNA denaturation was performed with 2N HCl/ 0.5%Triton X-100 solution for 30 min., followed by quenching with HCl (sodium tetraborate solution 0.1M pH8.5). Cells were incubated with FITC-conjugated anti-BrdU antibody (1/20; BD Biosciences), RNaseA (10 μ g/ml; Sigma), and propidium iodide (PI) (BD Biosciences) (20 μ g/ml). Stained nuclei from 10,000 cells were subjected to flow cytometry. Data were collected on a fluorescence-activated cell sorting (FACS) Canto II flow cytometer (BD Biosciences). Cell cycle analysis was

performed with BD FACS Diva software and FlowJo (FlowJo LLC, Ashland, OR). Experiments were performed in triplicates.

4.3.8 Apoptosis assay

NCI-H82 and NCI-H446 were seeded in 12-well plates at 0.5×10^6 cells per well. After 24h-treatment with PF-228 (1, 3, and $5 \mu\text{M}$) or DMSO, cells were stained with antibodies against cleaved Caspase-3 (1:50; Cell Signaling Technology) or, after 48h-treatment, with BrdU via TUNEL assay (APO-BrdU Kit; BD Biosciences) according to manufacturer's instructions. Staining was quantified by FACSCanto II. Data acquisition and analysis were performed with FlowJo. Experiments were performed in triplicates.

4.3.9 Wound healing assay associated with time-lapse video recording of cell motility

NCI-H196 and NCI-H446 were grown to confluence in 12-well plates. Cell monolayers were wounded using a micropipette tip and floating cells were washed off with PBS (Lonza). After overnight incubation, PF-228 or DMSO was added to culture medium. Cell movements within wounded area were monitored for 12h starting from the time drug was added using a Zeiss Axiovert 200M microscope (Zeiss, Thornwood, NY) at x200 magnification. Images were captured every 15-minutes from five different fields randomly selected in each well. About 100 individual cells were analyzed using the Tracking Analysis software. Individual cells were tracked manually using MTrackJ, an Image J (NIH, Bethesda, MD) plugin. Only non-dividing cells were analyzed to exclusively assess motility. Track's full length (LEN) was determined from the first point to the last point of the track and represented the distance covered by the cell during the experiment. Migration velocity was obtained by dividing LEN with experiment duration (12h). Experiments were performed in triplicates.

4.3.10 Matrigel invasion assay

Inserts separating the two chambers of 24-well invasion chambers (Corning, NY) were coated with Matrigel (0.3g/l) and incubated at 37°C for 2h. Lower chambers were filled with RPMI containing 10%-FBS. NCI-H196 and NCI-H446 were trypsinized, washed with PBS, suspended in 1%-FBS RPMI, plated in the upper chambers (25×10^3 and 10×10^4 cells per well for NCI-H196 NCI-H446, respectively), and incubated at 37°C for 3h. PF-228 (3 or $5 \mu\text{M}$) or DMSO was added in upper chambers 3h after seeding. After 12h incubation with the drug, cells remaining in the upper chamber were removed with cotton swabs and cells on the lower surface of the insert separating both chambers were fixed and stained with crystal violet. Image acquisition was performed with Axiovert 40 CFL Zeiss microscope (Carl Zeiss

Microscopy, LLC, Thornwood, NY). Images were obtained using ImageJ software (NIH, Bethesda, MD). Experiments were performed in duplicate.

4.3.11 Rac pull-down assay for activated GTPases

Active GTPases were pull-downed with Active Rac1 Pull-Down and Detection Kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's protocol. Cells were lysed with a lysis buffer containing a complete protease inhibitor cocktail. Equal amounts of proteins (800µg) were loaded into kit's pull-down columns. Samples were incubated with Rac/Cdc42 PAK1 PAK-binding domain and rocked for 1h. Agarose beads were collected by centrifugation (30 sec. at 6,000 *g* and 4°C), washed, resuspended with 50µl 2xSDS sample buffer, and boiled for 5 min. Proteins were resolved by 12% SDS-PAGE and electrotransferred onto a membrane probed with mouse anti-Rac1 antibody (Thermo Fisher Scientific). GTP loading controls were incubated with GTP-γS (0.1mM) for 0.5h at 30°C.

4.3.12 Statistics

Statistical analyses were performed using the statistical analysis software JMP Pro version 12 (SAS Institute Inc., Cary, NC). Multiple linear regression analysis was used for WST-1 and Chi square test of independence for cell cycle and apoptosis data. Descriptive statistics were reported as mean ± standard deviation. Significance level was set at $p < 0.05$ for each analysis.

4.4 Results

4.4.1 Pharmacological inhibition of FAK has several anti-tumoral effects in SCLC

To investigate whether FAK is involved in the aggressive phenotype of SCLC, we tested the changes of cellular phenotype induced by the FAK small-molecule inhibitor PF-228 in four SCLC cell lines (two growing in suspension: NCI-H82 and NCI-H146, an adherent: NCI-H196, and a mixed-morphology: NCI-H446).

4.4.1.1 *PF-228 inhibits FAK phosphorylation at Tyr397*

Treatment with increasing concentrations of PF-228 (0.1 to 10µM) decreased FAK phosphorylation (Tyr397) in all tested cell lines dose-dependently, without modifying total FAK expression (**Fig.1A**). Phospho-FAK (Tyr397) decrease was less important in the adherent cell line NCI-H196, even at higher drug concentrations (0.5-10µM versus 0.1-3µM).

4.4.1.2 PF-228 inhibits proliferation and progression through cell cycle in SCLC

Inhibition of FAK activity with 1 to 10 μ M PF-228 significantly decreased proliferation of the four SCLC lines dose-dependently ($p < 0.001$ for all tested concentrations beside 1 μ M in NCI-H196) (**Fig.38 B**). The effect was more pronounced in the suspension cell lines NCI-H82 and NCI-H146, which constitutively displayed higher proliferation rates. Cell cycle analysis showed that PF-228 inhibited progression through cell cycle by significantly reducing the S phase and inducing cell cycle arrest in G2/M phases in the four cell lines after 24h-treatment, dose-dependently ($p < 0.001$ for all tested concentrations) (**Fig.38 C**).

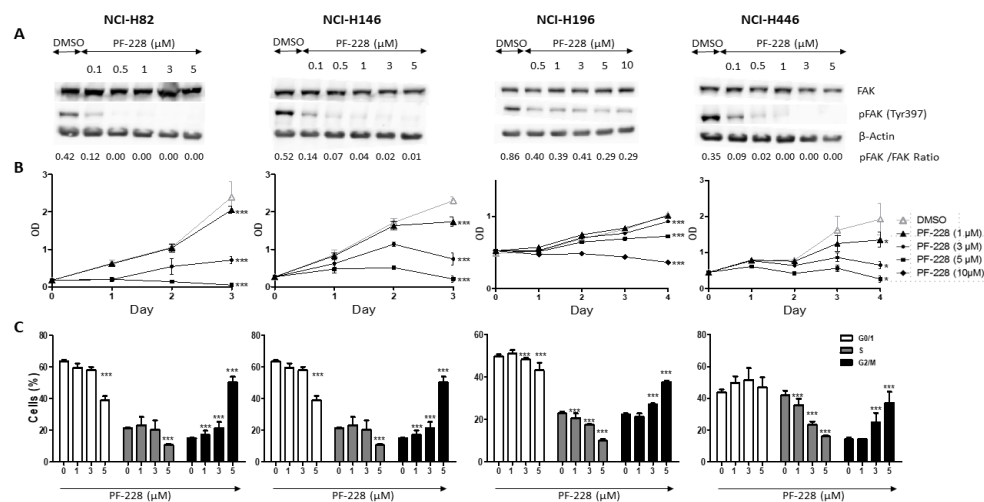


Figure 38: PF-573,228 (PF-228)'s effect on FAK expression/activity, cell proliferation, and cell cycle in SCLC cell lines.

A. FAK expression and phosphorylation evaluation by Western blot (WB). Whole cell lysates from four SCLC cell lines treated with PF-228 or DMSO control for 90 min. were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were incubated with antibodies against total FAK (125 kD), phospho-FAK (Tyr397) (125 kD), and β -Actin (45 kD) for normalization. Dose-dependent inhibition of FAK phosphorylation (Tyr397) was observed by WB in cell lines treated with PF-228 as compared to those treated with DMSO, while total FAK expression was not modified. WB densitometric quantification is available in Supplementary Fig.S1.

B. Cell proliferation evaluation by WST-1 assay. Four SCLC cell lines were cultured for three or four days in presence of PF-228 or DMSO. Dose-

dependent inhibition of proliferation was observed by WST-1 assay in cells treated with PF-228 as compared to those treated with DMSO. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean $\pm$ standard deviation (SD) (n=3). All the graphs represent one of three independent experiments with similar results. *** $P \leq 0.001$.

C. Cell cycle evaluation by flow cytometry. Four SCLC cell lines treated with PF-228 or DMSO for 24h were stained with anti-BrdU antibody and propidium iodide (PI), and the staining was quantified by fluorescence-activated cell sorting (FACS) analysis. Dose-dependent inhibition of DNA synthesis and induction of cell cycle arrest in G2/M phase was observed by flow cytometry in cell lines treated with PF-228 as compared to those treated with DMSO. Error bars represent mean $\pm$ SD from three independent experiments. *** $P \leq 0.001$.

4.4.1.3 *PF-228 induces apoptosis in SCLC*

PF-228 at concentrations of 1 to 5 μ M also significantly induced apoptosis in the four cell lines as demonstrated by a dose-dependent increase of PARP p85 expression by WB after 48h-treatment (**Fig.39 A**). This was confirmed by flow cytometry in NCI-H82 and NCI-H446 cell lines, which showed a significant increase of BrdU-positive and activated Caspase 3-positive cells after 48h-treatment ($p < 0.001$ for all tested concentrations except 1 μ M in NCI-H446 in the Caspase-3 assay) (**Fig.39 C**).

4.4.1.4 *PF-228 inhibits migration and invasion in SCLC*

Wound healing assay and modified Boyden chambers allowed the investigation of cellular migration and invasion in the two SCLC cell lines with an adherent component. PF-228 at a concentration of 3 μ M tended to decrease migration velocity from 5 to 2.5 μ m/min in NCI-H196 ($p = 0.0561$) and from 9 to 4 μ m/min in NCI-H446 ($p = 0.0916$) (**Fig.40 A**; Supplementary Video1). PF-228 also inhibited invasion after 12h-treatment at 3 μ M, with the number of invasive cells able to migrate to the lower side of the insert separating the two Boyden chambers decreasing from 150 to 50 per field (20x magnification) for NCI-H196 and from 45 to 5 per field for NCI-H446 (**Fig.40 B**).

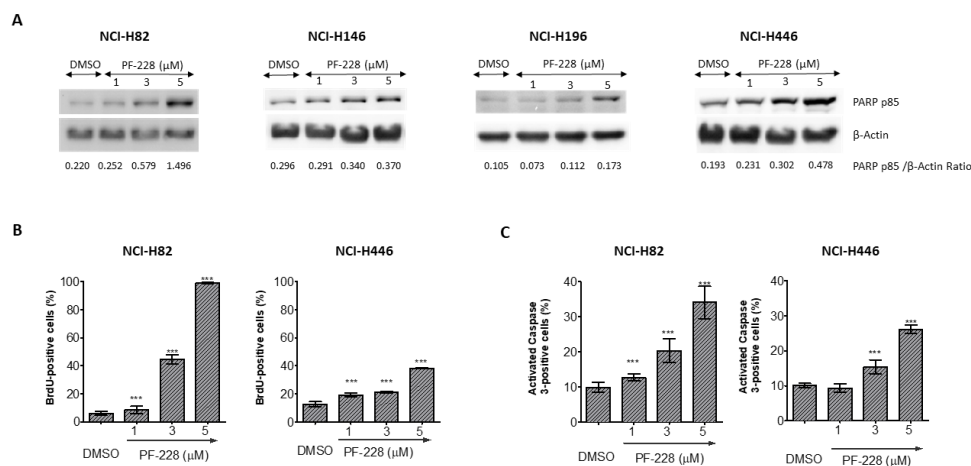


Figure 39: PF-228's effect on apoptosis in SCLC cell lines.

A. Apoptosis evaluation by PARP p85 WB. Whole cell lysates from four SCLC cell lines treated with PF-228 or DMSO for 48h were resolved by SDS-PAGE and blots were incubated with antibodies against PARP p85 (85 kD) and β-Actin (45 kD) for normalization. Dose-dependent increase of PARP p85 expression was observed by WB in cell lines treated with PF-228 as compared to those treated with DMSO. WB densitometric quantification is available in Supplementary Fig.S1.

B and C. Apoptosis evaluation by flow cytometry. Two SCLC cell lines treated with PF-228 or DMSO for 24h (B) or 48h (C) were stained with anti-BrdU antibody and PI (B) or Pacific-Cleaved Caspase 3 and PI (C), and the staining was quantified by FACS. Cells were first gated in PI channel (PI-A and PI-H) to discard cells debris and doublets. Dose-dependent increase of apoptotic cells (BrdU-positive cells (B) or activated Caspase 3-positive cells (C)) was observed by flow cytometry in cell lines treated with PF-228 as compared to those treated with DMSO. Error bars represent mean +/- SD from three independent experiments. *** $P \leq 0.001$.

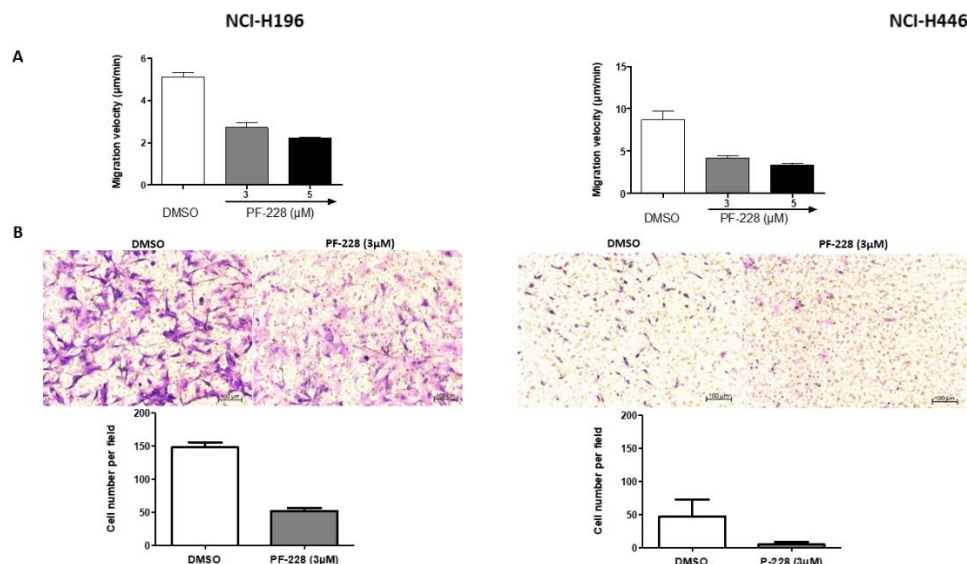


Figure 40: PF-228's effect on migration and invasion in SCLC cell lines.

A. Migration evaluation by wound healing assay associated with time-lapse microscopy. Two adherent SCLC cell lines were grown to confluence, wounded, incubated overnight with culture medium, and then treated with PF-228 or DMSO for 12h. Cells were monitored during these 12h using a Zeiss Axiovert 200M microscope (Zeiss, Thornwood, NY). Images were captured every 15min. Velocity of cell migration was measured using ImageJ. Decreased motility was observed in cell lines treated with PF-228 as compared to those treated with DMSO. Error bars represent mean $\pm$ SD from three independent experiments.

B. Invasion evaluation by modified Boyden Chamber assay. Two adherent SCLC cell lines (one adherent and one with mixed-morphology) were seeded on the top of an insert pre-coated with matrigel and separating the two chambers of a transwell. Culture medium containing 1%-FBS was placed in the upper chamber and 10%-FBS in the lower chamber. After 12h-treatment with PF-228 or DMSO, cells that moved through the pores towards the bottom of the insert were stained with crystal violet, digitally pictured, and quantified by the free software ImageJ (NIH, Bethesda, MD, USA). Right panels are pictures of SCLC cell lines stained with crystal violet on the lower side of the insert which are representative of the numerous fields (x10 magnification) analyzed in two independent experiments performed in duplicate wells. Left panels

represent quantification of the number of cells per field on the bottom of the insert. Decreased invasion was observed in cell lines treated with PF-228 for 12h as compared to those treated with DMSO. Error bars represent mean $\pm$ SD from two independent experiments.

4.4.1.5 *Inh14 and PF-271 also inhibit proliferation and induce apoptosis in SCLC*

To verify that PF-228's effects were related to FAK, we tested two other FAK inhibitors, Inh14 and PF-271, in NCI-H446. Similarly to PF-228, they both decreased FAK phosphorylation at Tyr397 and proliferation, and increased apoptosis as shown by increased PARP p85 expression (**Fig.41**).

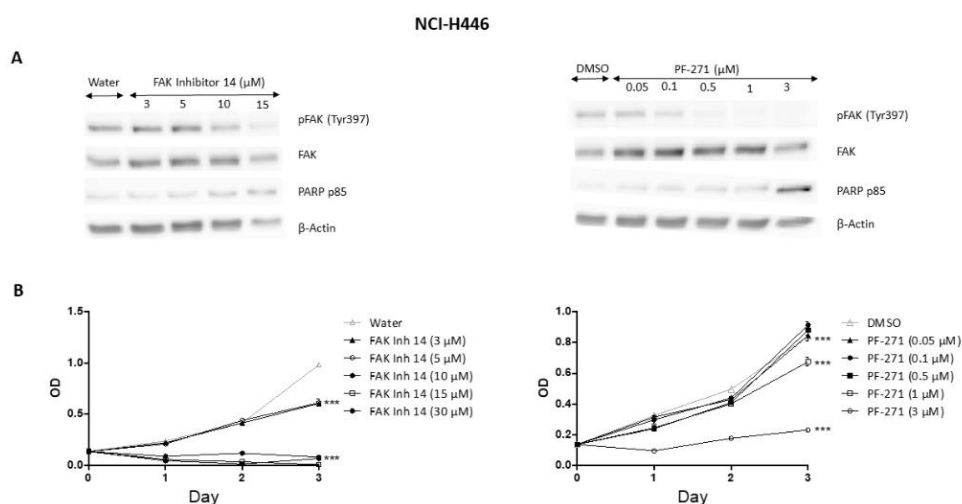


Figure 41: FAK Inhibitor 14 (Inh14) and PF-562,271 (PF-271)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H446 SCLC cell lines:

A. FAK expression and phosphorylation and PARP p85 expression evaluation by Western blot (WB). Whole cell lysates from NCI-H446 SCLC cell lines treated with Inh14, PF-271, or control for 60 min. (except 48h for evaluation of apoptosis via PARP p85 expression) were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were incubated with antibodies against total FAK (125 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β -Actin (45 kD) for normalization. Dose-dependent inhibition of FAK phosphorylation (Tyr397) and dose-dependent increase of PARP p85 expression, marker of apoptosis, was observed by WB in cell lines treated with Inh 14 or PF-

271 as compared to those treated with control, while total FAK expression was not modified.

B. Cell proliferation evaluation by WST-1 assay. NCI-H446 SCLC cell lines were cultured for three days in presence of Inh14, PF-271, or control. Dose-dependent inhibition of proliferation was observed by WST-1 assay in cells treated with Inh14 or PF-271 as compared to those treated with control. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean +/- standard deviation (SD) (n=3). All the graphs represent one of three independent experiments with similar results. *** $P \leq 0.001$.

4.4.2 Genetic inhibition of FAK leads to anti-tumoral effects only in presence of FRNK

4.4.2.1 *Loss of total FAK following FAK shRNA transduction does not affect proliferation and progression through cell cycle in SCLC*

Aiming to confirm the specificity of PF-228's anti-tumoral effects in SCLC cell lines, experiments were carried out in NCI-H82 and NCI-H446 cells where FAK was inhibited by a genetic approach, namely the stable transduction of FAK shRNA (five clones). WB confirmed the almost complete loss of total FAK and phospho-FAK (Tyr397) expression following transduction with FAK shRNA as compared with NT shRNA (**Fig.43 A1**). However, FAK shRNA transduction did not modify cell proliferation over three days and progression through cell cycle as evaluated by WST-1 and flow cytometry, respectively (**Fig.43 A2 and A3**).

Once again aiming to evaluate PF-228's specificity, we treated SCLC cell lines transduced with FAK or NT shRNA with PF-228. As expected, we observed a significantly less important inhibition of proliferation in cell lines transduced with FAK shRNA. We also showed that PF-228 induced apoptosis, as demonstrated by increased PARP p85 expression, only in cells transduced with NT shRNA (**Fig.42**).

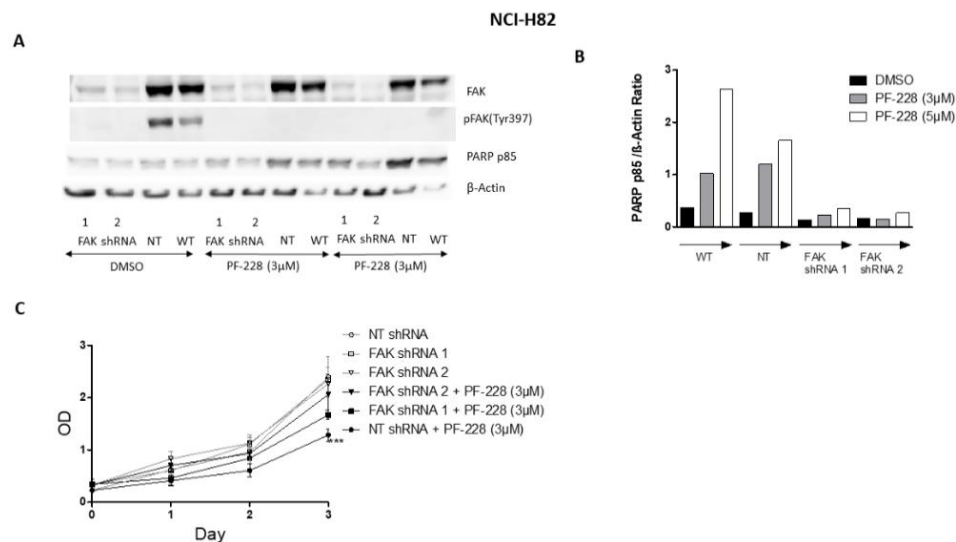


Figure 42: PF-573,228 (PF-228)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H82 SCLC cell lines transduced with FAK shRNA:

NCI-H82 SCLC cell lines were stably transduced with FAK shRNA or no-target (NT) shRNA as control, and submitted to puromycin selection for two weeks.

A. FAK expression/activation and PARP p85 expression evaluation by Western blot (WB). Whole cell lysates from NCI-H82 SCLC cell lines transduced with FAK or NT shRNA and treated with PF-228 or DMSO control for 90 min. (except 48h for evaluation of apoptosis via PARP p85 expression) were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were incubated with antibodies against total FAK (125 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β -Actin (45 kD) for normalization. Total FAK expression decreased in cell lines transduced with FAK shRNA but not in those transduced with NT shRNA, as compared to wild-type (WT) cell lines, independently of PF-228 treatment. Phospho-FAK expression was absent in cell lines transduced with FAK shRNA and those treated with PF-228, while it was expressed in cell lines transduced with NT shRNA and not treated with PF-228. PARP p85 expression, marker of apoptosis, significantly increased in cell lines transduced with NT shRNA and

treated with PF-228 but not in those transduced with FAK shRNA and treated with PF-228.

- B. WB densitometric quantification of PARP p85 expression. Densitometric quantification of PARP p85 expression on the WB displayed in Fig.42A was performed using the Quantity One analysis software (Bio-Rad). The Y-axis represents the level of each protein of interest normalized to β -Actin. The X-axis represents experimental conditions
- C. Cell proliferation evaluation by WST-1 assay. NCI-H82 SCLC cell lines transduced with FAK or NT shRNA were cultured for three days in presence of PF-228 or DMSO control. A significantly less important inhibition of proliferation was observed after PF-228 treatment in cell lines transduced with FAK shRNA as compared with those transduced with NT shRNA. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean $\pm$ standard deviation (SD) (n=3). All the graphs represent one of three independent experiments with similar results. *** $P \leq 0.001$.

4.4.2.2 *FRNK overexpression following transduction inhibits proliferation and survival in SCLC*

In order to address the apparent discrepancy between PF-228's effects and those of FAK shRNA transduction, we used a second genetic approach to inhibit FAK in NCI-H446, namely the stable transduction of a doxycycline-inducible FRNK vector. FRNK, which lacks FAK's N-terminal and kinase domains, is a known physical repressor of FAK signaling[202]. WB confirmed a significant and dose-dependent (doxycycline) increase of FRNK expression in NCI-H446 transduced with doxycycline-inducible FRNK vector and treated with doxycycline, as compared with those not treated with doxycycline or transduced with pCLX empty vector, while total FAK and phospho-FAK (Tyr397) expression remained unchanged (**Fig.43 B1**).

Interestingly, FRNK overexpression significantly decreased cell proliferation over five days ($p < 0.001$) and DNA synthesis after 48h-treatment with doxycycline ($p < 0.001$) as evaluated by WST-1 and flow cytometry, respectively (**Fig.43 B2 and B3**). FRNK overexpression also significantly induced apoptosis as shown by increased PARP p85 expression by WB after 48h-treatment with doxycycline (**Fig.43 B1**). The effects on proliferation, DNA synthesis, and apoptosis were proportional to doxycycline concentrations and FRNK expression levels. As opposed to FAK inhibition by FAK shRNA transduction, FAK inhibition by FRNK overexpression induced anti-tumoral effects similar to FAK pharmacological inhibition.

SD (n=5). All the graphs represent one of five independent experiments with similar results. *** $P \leq 0.001$.

3. Cell cycle evaluation by flow cytometry. SCLC cell lines were stained with anti-BrdU antibody and PI, and the staining was quantified by FACS. No significant difference in cell cycle was observed between cells transduced with FAK shRNA and those transduced with NT shRNA transfection. Error bars represent mean $\pm$ SD from three independent experiments. *** $P \leq 0.001$.

B. NCI-H446 cell lines were stably transduced with doxycycline-inducible FRNK-expression plasmid or empty vector (pCLX) as control, and submitted to blasticidin-selection for two weeks. Cells were treated with doxycycline for 48h before the experiments.

1. FAK and PARP p85 expression/activity evaluation by WB. Whole cell lysates from SCLC cell lines were resolved with SDS-PAGE and blots were incubated with antibodies against total FAK (125 kD), FRNK (41 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β -Actin (45 kD) for normalization. Significant increase of FRNK expression was confirmed by WB in SCLC cell lines transduced with FRNK and treated with doxycycline as compared to those not treated with doxycycline or transduced with pCLX empty vector. Significant increase of PARP p85 expression, a marker of apoptosis, was also observed by WB in cells expressing FRNK, while total FAK and phospho-FAK (Tyr397) expression remained unchanged.
2. Cell proliferation evaluation by WST-1 assay. SCLC cell lines were cultured for five days. Inhibition of proliferation was observed by WST-1 in cell lines expressing FRNK as compared to those not expressing it. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean $\pm$ SD (n=5). All the graphs represent one of five independent experiments with similar results. *** $P \leq 0.001$.
3. Cell cycle evaluation by flow cytometry. SCLC cell lines were stained with anti-BrdU antibody and PI, and the staining was quantified by FACS. DNA synthesis was decreased in cell lines expressing FRNK as compared to

those not expressing it. Error bars represent +/- SD from five independent experiments. *** $P \leq 0.001$.

4.4.2.3 FRNK overexpression following transduction in SCLC cell lines previously transduced with FAK shRNA inhibits proliferation and survival

Facing different results with the two genetic approaches used to inhibit FAK, we wondered whether the loss of FRNK was responsible for the absence of effect of FAK shRNA transduction on survival. To test this, we overexpressed FRNK in NCI-H446 cells stably transduced with FAK shRNA by transducing them with doxycycline-inducible FRNK vector. FRNK overexpression did not modify total FAK and phospho-FAK (Tyr397) expression, which were both downregulated by FAK shRNA transduction (**Fig.44 A**). However, in these double-transduced cells, with FAK shRNA and then FRNK, we observed an inhibition of cell growth over four days as evaluated by WST-1 ($p < 0.001$) (**Fig. 44 B**), and an induction of apoptosis as shown by increased PARP p85 expression by WB (**Fig. 44 A**). The effects on proliferation and apoptosis were both proportional to doxycycline concentrations and FRNK expression levels.

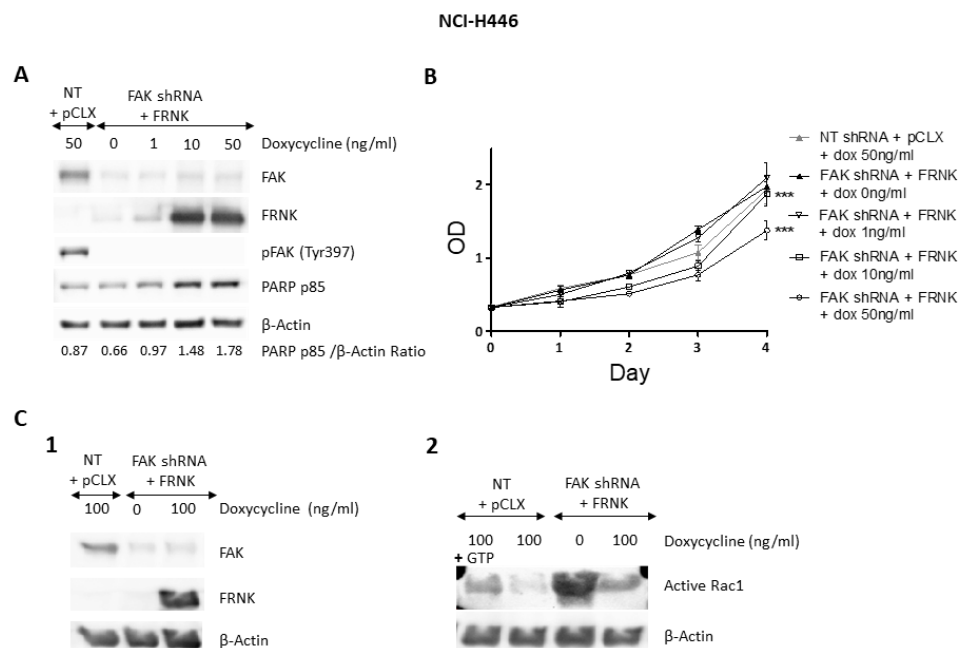


Figure 44: Effect of FRNK transduction on FAK expression/activity, proliferation, apoptosis, and Rac1 expression in SCLC cell lines transduced with FAK

shRNA. NCI-H446 cell lines were first stably transduced with FAK shRNA or no-target (NT) shRNA and then stably transduced with doxycycline-inducible FRNK-expression plasmid or pCLX empty vector as control. After transduction, they were submitted to puromycin and blasticidin-selection for two weeks. Finally, they were treated with doxycycline for 48h before the experiments.

- A. FAK and PARP p85 expression/activity evaluation by WB. Whole cell lysates from SCLC cell lines were resolved with SDS-PAGE and blots were incubated with antibodies against total FAK (125 kD), FRNK (41 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β -Actin (45 kD) for normalization. Decreased FAK and phospho-FAK (Tyr397) expression was observed by WB in cell lines double-transduced with FAK shRNA and FRNK as compared to those transduced with NT shRNA and PCLX. Increased FRNK expression was observed in cell lines double-transduced with FAK shRNA and FRNK when treated with doxycycline. Increased PARP p85 expression, a marker of apoptosis, was observed in cells expressing FRNK. WB densitometric quantification is available in Supplementary Fig.S2.
- B. Cell proliferation evaluation by WST-1 assay. SCLC cell lines were cultured for four days. Inhibition of proliferation was observed in cell lines double-transduced with FAK shRNA and FRNK and expressing FRNK after treatment with doxycycline. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean $\pm$ standard deviation (SD) (n=5). All the graphs represent one of five independent experiments with similar results. *** $P \leq 0.001$.
- C. Effects of FRNK on Rac1 activity. NCI-H446 SCLC cell lines were double-transduced with FAK shRNA and a doxycycline-inducible FRNK vector or with no-target (NT) shRNA and pCLX empty vector as control.
1. FAK and FRNK expression evaluation by WB. Whole cell lysates from SCLC cell lines were resolved with SDS-PAGE and blots were incubated with antibodies against total FAK (125 kD), FRNK (41 kD), and β -Actin (45 kD) for normalization. Decreased FAK expression was observed by WB in cell lines double-transduced with FAK shRNA and FRNK as compared to those transduced with NT shRNA and PCLX. Increased FRNK expression was observed in cell lines double-transduced with

FAK shRNA and FRNK when treated with doxycycline. WB densitometric quantification is available in Supplementary Fig.S1.

2. **Rac1 activation evaluation by Rac pull down assay for activated GTPases.** Whole cell lysates from SCLC cell lines were enriched in activated GTPases using a GST-PAK affinity assay (GTPases pull down assay). Enriched eluates were resolved with SDS-PAGE and blots were incubated with antibodies against Rac1 (21 kD) and β -Actin (45 kD) for normalization. In control cells double-transduced with NT shRNA and PCLX, WB revealed low activated Rac1 expression at baseline, while treating them with GTP significantly increased its expression. In cells double-transduced with FAK shRNA and FRNK, activated Rac1 expression was present in the absence of doxycycline, while doxycycline-induced FRNK expression significantly decreased its expression. WB densitometric quantification is available in Supplementary Fig.S2.

4.4.2.4 *FRNK keeps Rac1 GTPase inactivated in SCLC*

Based on a previous report in endothelial cells which also showed that different methods of FAK inhibition result in different functional outcomes and that this occurs through the regulation of Rac activation[479], we evaluated activated Rac1 level in NCI-H446 cell lines double-transduced with FAK shRNA and doxycycline-inducible FRNK vector using Rac pull-down assay for activated GTPases (**Fig.44 C1**). In NT shRNA and pCLX double-transduced cells used as control, with no FRNK expression, activated Rac1 level was low at baseline, while treating them with GTP significantly increased it as expected (**Fig.44 C2**). In cells transduced with FAK shRNA and doxycycline-inducible FRNK vector, activated Rac1 was present at baseline in cells without FRNK expression, while FRNK overexpression significantly decreased activated Rac1 level (**Fig.44 C2**). These results indicate that the loss of FRNK following the physical loss of total FAK increases activated Rac1 level. As Rac1 is a pro-proliferative protein[441,480], we have an explanation to why SCLC cell lines transduced with FAK shRNA remain proliferative (**Fig.43 A2**): the pro-proliferative effect of Rac1 activation counterbalances the anti-proliferative effect induced by the absence of FAK phosphorylation at Tyr397.

Since FAK is known to phosphorylate Paxillin and phospho-Paxillin to activate Rac1 via the adaptor protein CrkII[481], we evaluated phospho-Paxillin (Tyr118) expression in NCI-H446 by WB and immunofluorescence. The two methods showed that FAK inhibition, either with PF-228 or double-transduction with FAK shRNA and FRNK, did not modify phospho-Paxillin (Tyr118) expression, which was however low even at baseline (**Fig.45**).

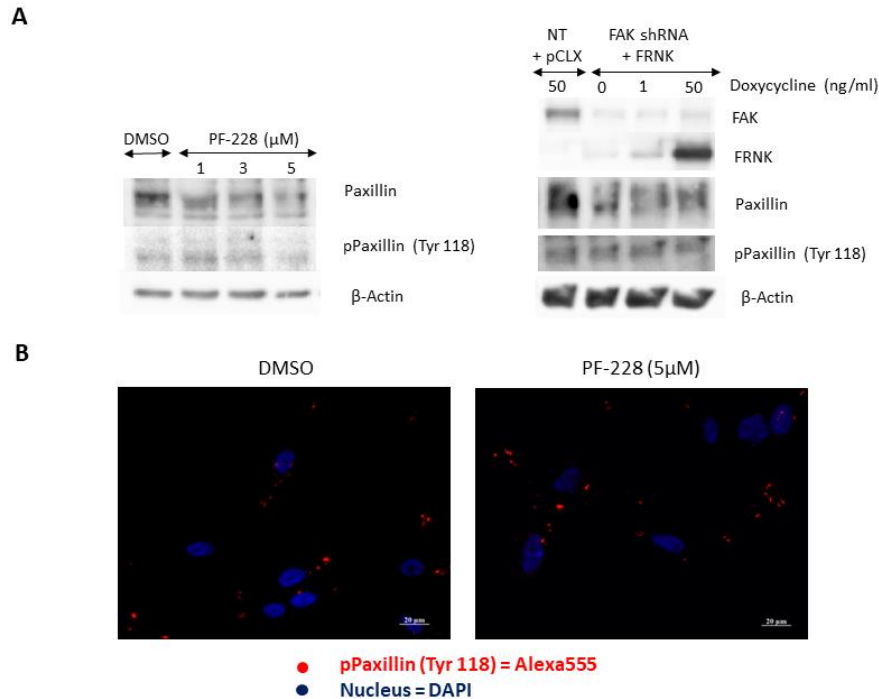


Figure 45: phospho-Paxillin expression in NCI-H446 SCLC cell line where FAK has been inhibited with pharmacological and genetic methods:

A. Phospho-Paxillin expression evaluation by WB. FAK was inhibited in NCI-H446 cell lines with PF-228 for 90 min. or a genetic method. In this last one, cell lines were first stably transduced with FAK shRNA or no-target (NT) shRNA and then stably transduced with doxycycline-inducible FRNK-expression plasmid or pCLX empty vector as control. After transduction, they were submitted to puromycin and blasticidin-selection for two weeks. Finally, they were treated with doxycycline for 48h before the experiments. Whole cell lysates from SCLC cell lines were resolved with SDS-PAGE and blots were incubated with antibodies against total FAK (125 kD), FRNK (41 kD), total Paxillin (68 kD), phospho-Paxillin (68 kD), and β -Actin (45 kD) for normalization. FAK inhibition, either with PF-228 or double-transduction with FAK shRNA and FRNK, did not modify phospho-Paxillin (Tyr118) expression.

B. Phospho-Paxillin expression evaluation by immunofluorescence (IF). NCI-H446 cell lines treated with PF-228 5 μ M or DMSO were formal-fixed, stained with an antibody against phospho-Paxillin (Tyr-118) (1/1000, rabbit polyclonal; Cell Signaling Technology), followed by an anti-

rabbit polyHRP secondary antibody, and revealed with tyramide Alexa555 (red). Nuclei were stained with DAPI (blue). FAK inhibition with PF-228 did not modify phospho-Paxillin (Tyr118) expression.

4.5 Discussion

In this study, we evaluated whether FAK, known to be overexpressed in SCLC tumors[195,199] and activated in SCLC cell lines[199], contributes to the aggressive behavior of SCLC and is a potential therapeutic target in SCLC. In a previous study, we showed that FAK was constitutively activated in SCLC cell lines, with high levels of FAK phosphorylation at Tyr397, and that the pharmacological inhibition of FAK with PF-228 decreased cell adhesion and modified cell phenotype[199]. Here, we explored the role of FAK in cellular functions relevant for cancer progression and showed for the first time that inhibition of FAK activity with PF-228 decreased proliferation, induced cell cycle arrest, increased apoptosis, and decreased motility and invasion. All these important anti-tumoral effects of PF-228 suggest that FAK is important in SCLC biology and may have a therapeutic potential. The inhibitory effect of PF-228 on SCLC motility and invasion was similar to the results reported in other cancer types or in normal cells[219]. Of note, we tested migration and invasion only in the two adherent cell lines as these functions are difficult to evaluate in suspension cell lines. Interestingly, we observed an effect of PF-228 on proliferation and apoptosis already at low drug concentrations. The first study which tested PF-228 showed an effect on migration and focal adhesion turnover but failed to demonstrate an effect on proliferation and survival in prostate cancer cell line PC3, fibroblastic cell line REF52, and canine kidney cell line MDCK[219]. Another study showed that PF-228 inhibited proliferation and induced apoptosis in endometrial cancer cell lines but, as opposed to our study, much higher concentrations of PF-228 were used (50 μ M). The fact that low concentrations of PF-228 inhibited proliferation and survival in SCLC cell lines suggest the specificity of the drug and the importance of FAK in pro-proliferative and pro-survival signaling pathways. Other FAK inhibitors induced inhibition of proliferation or survival *in vitro* in various cell types but were not specific of FAK (e.g.: TAE-226 inhibits FAK, PYK2, and IGF-1R)[482-484]. In this study, we additionally tested two other FAK inhibitors, Inh14 and PF-271[223,485], and observed that they also inhibited proliferation and induced apoptosis in SCLC. This strengthened us in the idea that PF-228's effects were related to FAK, even though Inh14 and PF-271 are both less FAK-specific than PF-228, known to have the highest FAK-specificity among FAK inhibitors.

In order to better address the specificity of PF-228's effects on proliferation and survival in SCLC cell lines, we evaluated the consequence of FAK inhibition by a genetic method, namely FAK shRNA stable transduction. Surprisingly, the physical loss of FAK did not impact on proliferation and cell cycle. But interestingly, treatment

of FAK shRNA-transduced cells with PF-228 did not induce apoptosis and had only a limited effect on proliferation. The Absent/limited effect of PF-228 in cells with no/low FAK expression also suggests the drug's specificity.

To address the apparent discrepancy between PF-228's effects and those of FAK shRNA transduction, we used a second genetic approach to inhibit FAK, namely the stable transduction of doxycycline-inducible FRNK vector leading to the overexpression of FRNK, a truncated protein including only FAK's carboxy-terminal non-catalytic domain and a well-known physical repressor of FAK signaling[202,456]. We observed that, as with PF-228, FRNK overexpression inhibited cell proliferation and DNA synthesis and increased apoptosis in SCLC cell lines. At this step, we hypothesized that the opposite results obtained with the two genetic approaches we used to inhibit FAK were related to FRNK, absent in cells transduced with FAK shRNA while present in those transduced with doxycycline-inducible FRNK vector and expressing FRNK. Our hypothesis was confirmed in double-transduced SCLC cell lines, first with FAK shRNA and then with FRNK, which revealed anti-tumoral effects in cells overexpressing FRNK. In a similar way, it has previously been reported that different methods of FAK inhibition result in different functional outcomes in endothelial cells: approaches inhibiting FAK phosphorylation at Tyr397 (such as FAK small-molecule inhibitors or FRNK transduction) inhibited proliferation and migration, while those abolishing FAK expression (such as FAK shRNA or siRNA) did not impact on these cellular processes[479]. Also supporting the importance of FRNK in the regulation of proliferation, a previous report showed that expressing FRNK with a C1034S mutation disrupted focal adhesion binding but had no effect on proliferation[302].

In endothelial cells, FAK has been proposed as a phospho-regulated repressor of the activation of Rac[479], a pro-proliferative GTPase present in focal adhesions[441,486]. This was based on the observation that FRNK expression, FAK Tyr397F mutation (simple substitution of Tyr397 with a non-phosphorylated residue), or treatment with a FAK kinase inhibitor decreased Rac activation induced by complete growth medium, while the physical loss of FAK following FAK shRNA transduction did not affect it[479]. Similarly, in cells double-transduced with FAK shRNA and doxycycline-inducible FRNK vector, we found high level of activated Rac1 in cells overexpressing FRNK, while it was low in the absence of FRNK expression. Based on these results, we propose the following model in SCLC, schematized in Fig.6: 1/ In normal conditions (absence of FAK inhibition) in SCLC, FAK constitutive activation results in FAK phosphorylation at Tyr397, leading to the activation of downstream phosphorylation-dependent signaling and to changes in the conformation of FAK and/or its binding partners, which allow Rac1 activation in the focal adhesion complex. 2/ PF-228 and FRNK overexpression both inhibit FAK phosphorylation at Tyr397, leading to the inhibition of downstream signaling and the

absence of change in conformation of FAK and/or its binding partners, which prevents Rac1 activation. This results in anti-tumoral effects, as observed in our experiments. 3/ In contrast, the physical loss of FAK after FAK shRNA transduction induces an inhibition of FAK phosphorylation-dependent signals but allows the activation of Rac1 because of the absence of repression by FAK. This last event results in pro-tumoral effects counterbalancing the anti-tumoral effects of FAK phosphorylation inhibition, explaining why FAK shRNA transduction did not affect proliferation and survival in the SCLC cell lines we tested.

Altogether our results suggest that, in order to induce proliferation and survival in SCLC cell lines, the physical presence of FAK is not required because the physical loss of FAK release the repressive signal on Rac and allows its activation, which induces proliferation and survival. In contrast, when the FRNK region of the FAT domain is present, FAK phosphorylation at Tyr397 seems necessary to induce proliferation and survival. Importantly, in a natural setting, there is no FAK shRNA; normal or cancer cells express total FAK (including FRNK) and FAK phosphorylation at Tyr397 is required for their proliferation and survival. Therefore, we can conclude that FAK plays an important role in various pro-tumoral properties of SCLC through its kinase domain and that inhibiting FAK phosphorylation at Tyr397 may have a therapeutic potential. Even though the discoveries made with FAK shRNA correspond to an artificial setting, they suggest that FAK small-molecule inhibitors should target the kinase domain but not FAK's regions which play a repressive role on pro-proliferative proteins, such as FRNK on Rac. Recently, small-molecule inhibitors targeting different FAK scaffolding protein-protein interactions have been developed and shown to induce anti-tumoral effects in preclinical studies[476], but further development of such inhibitors should take into account the complexity of FAK in order to be successful.

Of note, we did not find any phospho-Paxillin (Tyr118) expression modification in SCLC cells where FAK was inhibited with PF-228 or FAK shRNA +/- FRNK transduction. Since FAK is known to phosphorylate Paxillin and phospho-Paxillin to activate Rac1 via the adaptor protein CrkII[481], we expected to find an inhibition of Paxillin phosphorylation following FAK inhibition. However, similarly to our observation, previous studies also reported that FAK did not affect Paxillin tyrosine phosphorylation level[459,487]. Further investigations are required to better understand these observations.

To be mentioned, while PF-228 induced cell cycle arrest in G2 and S phases, FRNK transduction induced cell cycle arrest in S phase only, which was however sufficient to impact on proliferation and apoptosis. We assume that this discrepancy is related to an off-target effect of PF-228, which often happens with small-molecule inhibitors, even the specific ones. Nevertheless, this does not change the conclusion that FAK

plays a role in SCLC proliferation and survival since we showed that PF-228 and FRNK transduction both inhibited cell proliferation and induced apoptosis in SCLC cell lines.

More in depth investigation of FAK's role in cell cycle, apoptosis, and specifically DDR in SCLC may be relevant. Indeed, a recent study showed that FAK regulates DDR and that FAK inhibition by PF-271, RNA interference, or CRISPR/CAS9 gene editing induces persistent DNA damage and radiosensitizes KRAS-mutated NSCLC cell lines and xenografts[374]. In parallel, another study showed that nuclear FAK stimulates gene transcription favoring DDR and that FAK ablation by CRISPR/Cas9 editing induces DNA damage and increased radiosensitivity in NSCLC cells[373]. Similarly, it has recently been demonstrated that FAK overexpression is a radioresistance biomarker in locally-advanced HPV-negative head and neck squamous cell carcinoma (HNSCC), also a smoking-related malignancy, and that its inhibition with PF-271 radiosensitized HNSCC cell lines, with increased G2/M arrest and DNA damage[386]. In this context, combining FAK small-molecule inhibitor with radiotherapy in SCLC certainly deserves further investigations.

In summary, experiments using PF-228, a FAK small-molecule inhibitor, showed that inhibition of FAK phosphorylation at Tyr397 decreased proliferation, induced cell cycle arrest, increased apoptosis, and decreased motility and invasion in SCLC cell lines. FAK inhibition by FRNK overexpression after transduction of a doxycycline-inducible FRNK vector also induced inhibition of proliferation and survival, suggesting the specificity of PF-228. In contrast, FAK inhibition by FAK shRNA transduction did not affect proliferation and survival, probably because the physical absence of FRNK released a repressive signal on Rac, a pro-proliferative protein. Taken collectively, these data demonstrate that FAK is important in SCLC biology and that targeting its kinase domain may have a therapeutic potential in SCLC, while targeting its FAT domain should be avoided or done carefully to prevent pro-proliferative proteins from counter-balancing the anti-tumoral effects of FAK inhibition. Further studies in SCLC xenograft models are required to better understand the complexity of FAK in SCLC. Ultimately, this may lead to the evaluation of FAK inhibitors in clinical trials of patients suffering from SCLC, a deadly disease which still lacks efficient targeted therapies.

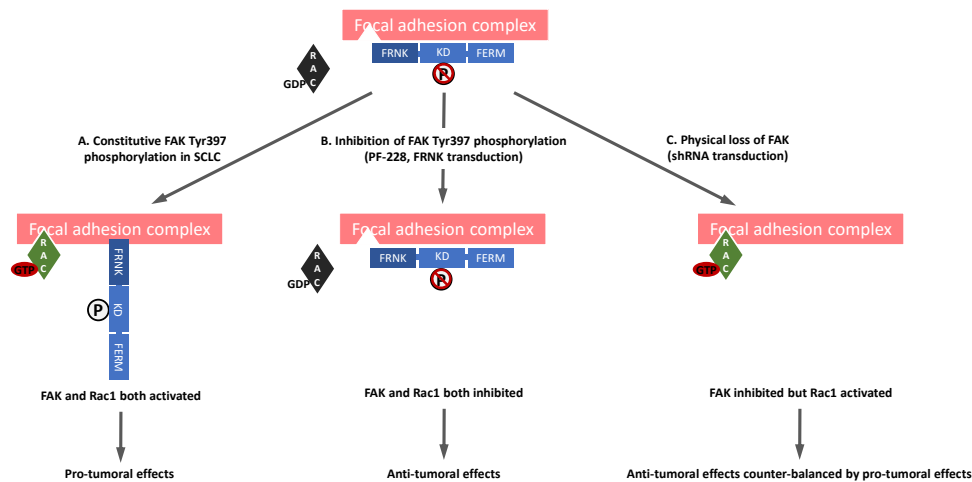


Figure 46: A model of FAK as a regulator of Rac1 activation in SCLC.

- A. "Normal" conditions in SCLC with FAK constitutively activated. FAK phosphorylation at Tyr397 leads to the activation of downstream phosphorylation-dependent signaling and to changes in the conformation of FAK and/or its binding partners, which allow Rac1 activation in the focal adhesion complex. These two events result in pro-tumoral effects.
- B. FAK inhibition by PF-228 and FRNK overexpression. These two methods inhibit FAK phosphorylation at Tyr397, leading to the inhibition of downstream signaling and the absence of change in conformation of FAK and/or its binding partners, which prevents Rac1 activation. These two events result in anti-tumoral effects.
- C. FAK inhibition by FAK shRNA transduction. The physical loss of FAK induces an inhibition of FAK phosphorylation-dependent signals but allows the activation of Rac1 because of the absence of repression by FAK. The anti-tumoral effects related to the absence of FAK are counterbalanced by the pro-tumoral effects related to Rac1 activation.

5 CONCLUSIONS, LIMITATIONS AND FUTURE PERSPECTIVES

5.1 Conclusions

SCLC is a highly aggressive tumor with a remarkable initial sensitivity to platinum and etoposide-based chemotherapy and radiation therapy [62,63,147]. However, despite high initial responses to chemotherapy +/- radiation therapy, most patients will experience recurrent disease and limited efficacy of further-line treatments, resulting in poor survival [68,73,75,81]. New therapeutic strategies focusing on different pathways within cancer cells and tumor microenvironment that improve the durability of responses to front-line therapy and have activity after disease recurrence are needed.

Targeting FAK, which is as an important regulator of biological processes involved in SCLC aggressive behaviour (for instance cell growth, survival, adhesion, spreading, migration, invasion, angiogenesis, DNA damage repair, radioresistance, and tumor immune evasion), appears as highly relevant in this disease. Aiming to improve the understanding of SCLC biology with the ultimate goal of improving its poor prognosis, and based on preliminary data suggesting that FAK activation plays a role in the invasive behaviour of SCLC, we conducted two studies.

In the first study, we showed by mIF-IHC that FAK and phospho-FAK are both significantly overexpressed in lung cancer as compared to normal lung tissues. Interestingly, we also showed that, among lung cancers, FAK and phospho-FAK expression, as well as the ratio between phospho-FAK and FAK expression, are significantly higher in SCLC compared to NSCLC. Moreover, we validated these observations by WB of NSCLC, SCLC, and normal lung tissue lysates. However, we did not find any correlation between FAK and activated FAK expression in lung cancer and RFS or OS in NSCLC and SCLC patients.

In the second study, experiments using FAK small-molecule inhibitors (PF-573,228, PF-562,271, FAK Inhibitor 14) showed that inhibition of FAK phosphorylation at Tyr397 decreased proliferation, induced cell cycle arrest, increased apoptosis, and decreased motility and invasion in SCLC cell lines. FAK inhibition by FRNK overexpression after transduction of a doxycycline-inducible FRNK vector also induced inhibition of proliferation and survival, confirming the specificity of FAK small-molecule inhibitors. In contrast, FAK inhibition by FAK shRNA transduction did not affect proliferation and survival, probably because the physical absence of FRNK released a repressive signal on Rac, a pro-proliferative protein. Taken collectively, these data demonstrated that FAK is central in SCLC biology and that targeting its

kinase domain may have a therapeutic potential in SCLC, while targeting its FAT domain should be avoided or done carefully to prevent pro-proliferative proteins from counter-balancing the anti-tumoral effects of FAK inhibition. Further studies in SCLC xenograft models are required to better understand the complexity of FAK in SCLC. Ultimately, this may lead to the evaluation of FAK inhibitors in clinical trials of patients suffering from SCLC, a deadly disease which still lacks efficient targeted therapies.

5.2 Limitations

Our study has some limitations. First, our data suggesting the antitumoral effect of FAK inhibition in SCLC have been generated in SCLC cell lines, which may not reflect the complex molecular biology of this aggressive disease in human tissues. This explains why several inhibitors with promising results in preclinical studies failed in clinical trials. For instance, PARP inhibitors targeting DDR genes and proteins highly expressed and activated in SCLC provided an antitumoral activity in preclinical studies. Based on analysis of tumor biopsy prior to the initiation of first-line treatment, patients enrolled in several studies including the association of the PARP inhibitor olaparib and the anti-PD-L1 durvalumab in a phase II trial [372], as well as a phase II trial with the PARP inhibitor veliparib combined with or without the chemotherapy agent temozolomide in recurrent SCLC did not meet efficacy criteria [371]. Anti-angiogenic agents (**table 1**) and anti-DLL-3 (Rova-T) also failed to significantly improve outcomes in SCLC, while preclinical data were promising [61,185].

Second, FAK and p-FAK staining was performed in tumor samples from treatment-naïve patients and may not represent modification of FAK expression and activity at progression as well as in refractory disease. Additionally, in our study, only nine frozen SCLC samples were available for WB analysis (**Fig.37**). These nine frozen samples were not paired with FFPE SCLC samples. Altogether, the correlation of WB (**Fig.37**) and mIHC (**Fig.35**) analysis was not feasible. The confirmation of WB analysis will be performed with a larger sample size tumors

Third, FAK FISH analysis has been not performed and correlated with protein expression in this work. In NSCLC, it has been shown that oncogenic drivers amplification such as MET and HER2 at gene level, evaluated by FISH is correlated with protein level [488-492]. Importantly, FISH gene (MET,HER2) amplification has been correlated with better ORR when treated with targeted therapy[490,493]. Nevertheless, FAK FISH amplification (FAK/CEP8 ratio>1.8) has been reported both in SCLC cell lines and 17% of 46 SCLC primary tumor and are correlated with FAK overexpression at mRNA and protein level [199].

5.3 Future perspectives

Based on the data we obtained in this work, we strengthened our hypothesis that FAK activation plays a key role in the invasive behaviour of SCLC, and that FAK may represent a good target for anti-cancer therapeutic interventions in SCLC in combination with standard therapies such as platinum-based chemotherapy, radiochemotherapy, or immunotherapy. Association of FAK inhibitors with these standard treatments may have synergistic effects and improve outcomes of SCLC patients (**Fig 47**).

We propose the following five future directions to investigate further the therapeutic role of FAK inhibition in SCLC. To achieve this, we will collect human tissue samples from patients with newly diagnosed ES-SCLC prior to initiating treatment, as well as at the time of progression or recurrence. These samples will be used in an orthotopic SCLC patient-derived xenograft (PDX) mouse model to facilitate comprehensive molecular studies, including genome transcriptome and (phospho)proteomic profiling (**Fig 47**).

Future direction 1: To evaluate the mechanisms responsible for FAK overexpression and activation and the role of FAK as potential mechanism of primary and acquired resistance to chemotherapy and/radiation in SCLC (**Fig 47**). For instance, how biallelic loss of TP53 and RB1 as well as SCLC subtypes (ASCL1, NEUROD1...) may modulate both FAK expression and activity will be investigated. Of note, it has been shown that nuclear FAK functions as a scaffold for TP53 and MDM2 in a kinase-independent manner, increasing TP53 polyubiquitylation and degradation [306]. We will perform TP53, Rb1, FAK next generation sequencing and protein quantification by IHC. Inactivation of TP53 and/or Rb1 will be correlated with FAK expression and activity. SCLC characterisation by mRNA level of ASCL1, NEUROD1, POU2F3 and YAP1 will be correlated with FAK expression and activity. Alterations correlation correlated with modification of FAK expression and activity will be confirm by in vitro experiments that overexpress or knock down the related gene (CRISPR- genome-editing, shRNA).

Future direction 3: To investigate the potential role of FAK in SCLC resistance to immunotherapy with PD-1 and PD-L1 ICIs in a genetically-engineered mouse model of SCLC (conditional triple knockout Trp53/ Rb1/Rbl2). The hypothesis is that genetically-engineered mice treated with a FAK inhibitor will be more sensitive to PD-1 or PD-L1 ICIs. It has been shown that some oncogenic abnormalities are correlated with refractoriness to ICIs [494], such as EGFR mutations, HER2 amplifications, MET activations, ROS1, ALK, and RET rearrangements [495,496]. For instance, HER2 negatively regulates stimulator of interferon genes (STING) signaling [497]. Similarly, loss of phosphatase and tensin homolog (PTEN) and

activation of the PI3-kinase pathway in cancer cells can also promote resistance to ICIs and has been linked to increased PD-L1 expression and immunoresistance in human glioma [498]. Additionally, FAK has been shown to induce the expression of inflammatory genes that inhibit antitumor immunity in the microenvironment in squamous cell carcinoma from mouse skin carcinomas. Nuclear FAK–IL-33 complex interacted with a network of chromatin modifiers and transcriptional regulators, including TAF9, WDR82, and BRD4, which promote the activity of nuclear factor kB (NF-kB) and its induction of genes encoding immunosuppressive chemokines, including CCL5 [362,425,499]. In pancreatic ductal adenocarcinoma, FAK activation has been shown to induce high number of tumor-associated immunosuppressive cells and a fibrotic stroma that functions as a barrier to T cell infiltration [278].

Future direction 4: To investigate the signaling events downstream of FAK responsible for the main phenotypic changes observed in our data. In order to document the effects of the FAK inhibition on a large number of phosphorylation events, which are otherwise difficult to characterize, we will use a high-throughput label-free phosphoproteomic mass spectrometry approach after the highly selective, reproducible, and sensitive titanium-IMAC (Ti4+-IMAC: immobilized metal ion affinity chromatography) based phosphopeptide enrichment method. The hypothesis is that a better understanding of the FAK signaling pathways will help us to predict potential resistance or bypass pathways to FAK TKIs.

Future direction 5: To assess the potential of FAK expression/activation as a predictive biomarker of response (theranostic) to FAK TKI, which may be evaluated in translational clinical trial in SCLC based on the results of the mentioned researches. We will evaluate the antitumoral potential of FAK inhibition in an orthotopic SCLC PDX mouse model, as well as the predictive value of FAK and phospho-FAK expression in these SCLC tumors. The hypothesis is that FAK inhibition will decrease lung tumor growth and metastases and that SCLC PDX mice with increased FAK and phospho-FAK expression will benefit more from FAK inhibition (**Fig 47**). Fresh tumor samples at diagnosis and at recurrence or progression will be transplanted and expanded in nude mice. Mice PDX will be treated by FAK TKI or placebo. ORR will be evaluated according FAK, p-FAK expression (evaluated by mIHC) and FAK gene amplification (assessed by FISH). FAK, phospho-FAK and FAK FISH could therefore be use as predictive biomarker in early clinical trial in human.

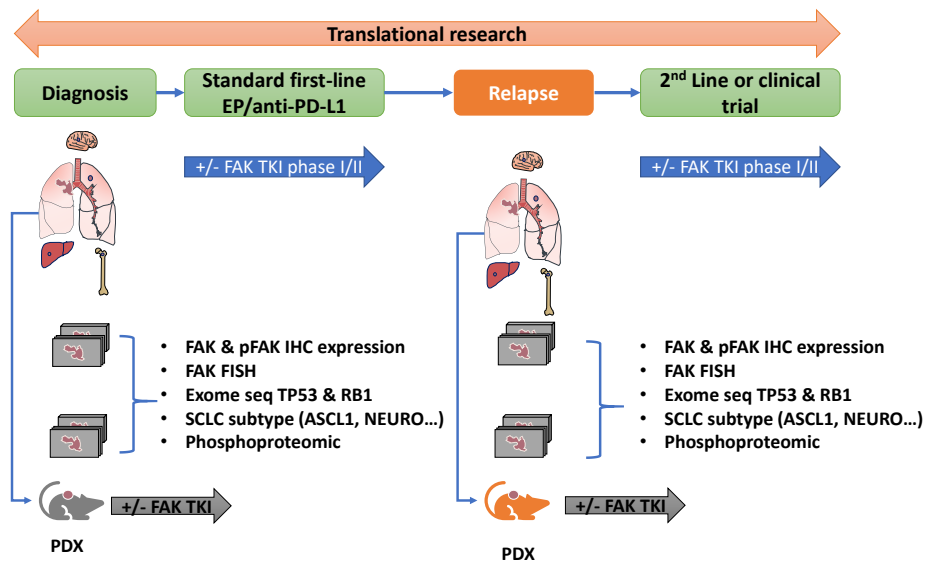
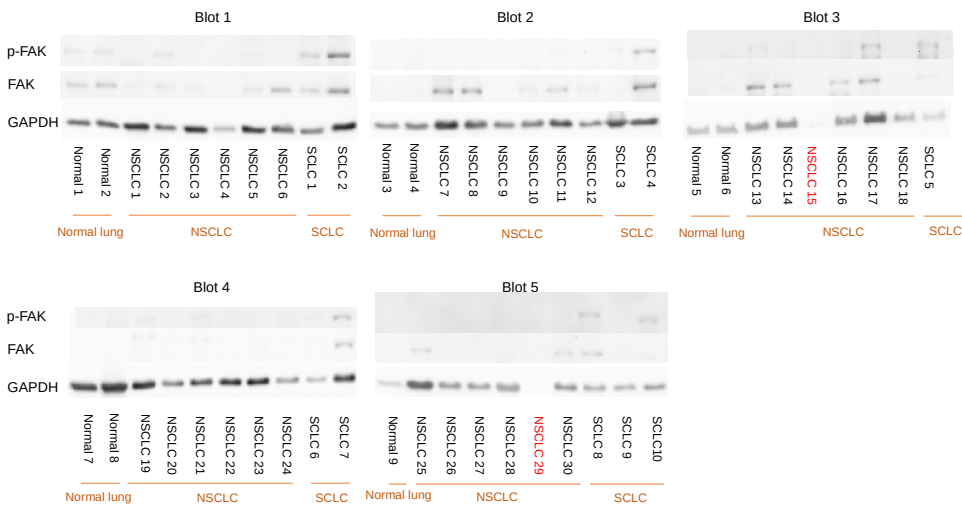


Figure 47: Overall design of a potential translational research evaluating the safety and efficacy of a FAK TKI in a SCLC clinical trial, with associated preclinical studies, investigation of FAK & pFAK as a potential predictive biomarkers of FAK TKI efficacy as well as of FAK role in the mechanism of primary and acquired resistance to standard first and further-line therapies in SCLC.

6 SUPPLEMENTARY DATA

The Figure 37 can be completed by these supplementary data, which represent WB performed in the study related to the increased expression and activation of FAK in small-cell lung cancer compared to non-small-cell lung cancer (chapter 3)



Supplementary data 1: WB of FAK and phospho-FAK (Y397) expression in normal lung, NSCLC, and SCLC tissue lysates.

The Figures 38A, 39A, 43A, 43B, and 44A can be completed by these supplementary data, which represent the quantification of WBs displayed in the study related to the therapeutic potential of focal adhesion kinase inhibition in small cell lung cancer (chapter 4).

Fig. 38A WB quantification

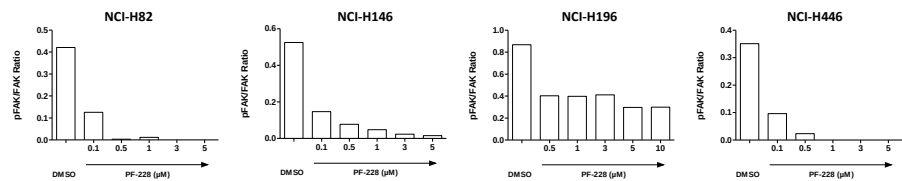


Fig. 39A WB quantification

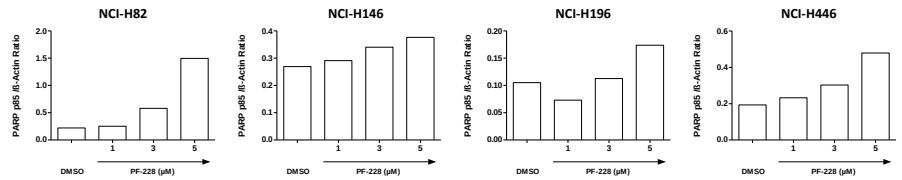


Fig. 43 A WB quantification

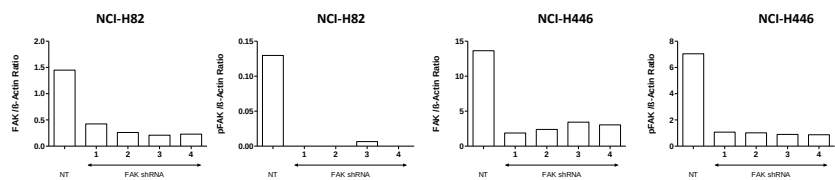


Fig. 43 B WB quantification

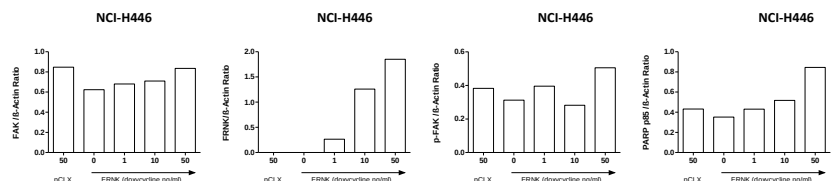
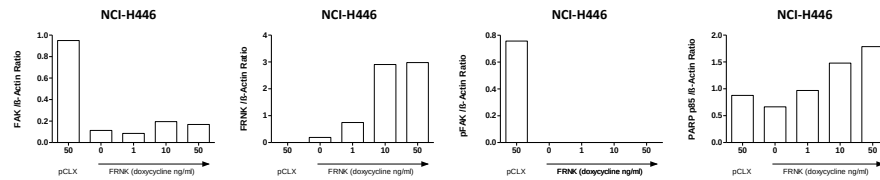


Fig. 44 A WB quantification



Supplementary data 2: The Y-axis represents the level of protein of interest normalized to β -Actin. The X-axis represents experimental conditions. These graphs confirm the conclusions drawn in the manuscript based on the WB pictures.

7 FIGURES

Figure 1: Estimated global numbers of new cancer cases with proportions worldwide, both sexes combined, in 2018. The area of the pie is proportional to the number of new cases (Globocan 2018 – <http://gco.iarc.fr>). 16

Figure 2: Estimated global numbers of cancer-related deaths with proportions worldwide, both sexes combined, in 2018. The area of the pie is proportional to the number of deaths (Globocan 2018 – <http://gco.iarc.fr>). 17

Figure 3: Combined estimates of relative risk for lung cancer according to smoking status and radon concentration exposure. 18

Figure 4: Relative risk of major histological types of lung cancer according to residential radon concentration exposure. 19

Figure 5: Schematic showing the location of the different cell types constituting the epithelial surfaces of the airways. Basal cells of the submucosal glands as well as central compartment are suggested to give rise to squamous cell carcinomas. Cells from peripheral airway compartment (type 2 pneumocyte) give rise to adenocarcinomas. Small-cell lung cancers and neuroendocrine tumors (NETs) arise from neuroendocrine cells located both in proximal compartment and submucosal glands. 20

Figure 6: Molecular classification of lung cancer. In non-small-cell lung cancer, previously classified by histological features only, a molecular classification has recently emerged, which determines tumor behavior and response to therapy. Small-cell lung carcinomas, squamous cell carcinomas, and adenocarcinomas harboring KRAS and STK11 mutations are associated with high mutation burden related to tobacco smoking. These lung cancers associated with smoking are genetically complex tumors as opposed to adenocarcinomas occurring in never smokers, which usually harbor an oncogenic driver targetable with a small-molecule tyrosine kinase inhibitor (TKI) such as EGFR and ALK TKIs. 21

Figure 7: Frequency of molecular alterations in six genes in A: 18.679 NSCLC samples and B: 384 NSCLC samples from never smokers. Data are presented as percentages. 23

Figure 8: Management strategies of non-small-cell lung cancer according to disease stage. 24

Figure 9: Management of small-cell lung cancer according to disease-stage. 25

Figure 10: Treatment options in extensive-stage SCLC. Standard treatment in bold. 27

Figure 11: Genomic alterations in small-cell lung cancer. Adapted from [148]. 33

Figure 12: Major genetic events and their roles in SCLC development and progression (from [159]). A model of SCLC development in the Rb/p53 conditional-mutant mice. Pulmonary neuroendocrine cells are likely cells-of-origin. Validated or potential tumor suppressors and oncogenic drivers are indicated in blue and red, respectively. 34

Figure 13: Signaling pathways recurrently affected in SCLC. Red and blue boxes denote genes with activating and inactivating alterations, respectively. Deep blue boxes highlight the bi-allelic inactivation of TP53 and RB1. Genes found expressed at high levels are shown in red font. Adapted from [148]. 35

Figure 14: Proportion of small-cell lung cancer molecular subtypes, defined based on the expression of the following key transcription regulators: achaete-scute homologue 1 (ASCL1) for the classic subtype; neurogenic differentiation factor 1 (NeuroD1) for the variant subtype; POU class 2 homeobox 3 (POU2F3) and yes-associated protein 1 (YAP1) for the non-neuroendocrine subtype. 36

Figure 15: Achaete-scute homologue 1 (ASCL1) small-cell lung cancer subtype. Notch signaling is activated by the binding of a Notch receptor to the extracellular domain of a Notch ligand (DLL, JAG) expressed on the SCLC cell surface. Notch receptor binding with its ligand generates cleavage of the Notch receptor, resulting in release of the Notch intracellular domain (NICD) which promotes ASCL1 inhibition through HES/HEY 1. Most SCLCs harbor Notch inactivation or ASCL1 overexpression which leads to DLL3 overexpression on the cell surface in approximately 70% of SCLCs. Adapted from [181,183,185,188] 38

Figure 16: The focal adhesion kinase (FAK) domain structure. The protein band 4.1-ezrin-radixin-moesin (FERM) homology domain on the amino-terminal side. The kinase domain indicates the region of catalytic activity. PR1 and PR2 denote proline-rich regions 1 and 2 in the carboxyl terminus. Important tyrosine phosphorylation (P) sites are indicated; Y397, K454 and H58 have crucial roles in FAK activation. FAT denotes the focal adhesion targeting domain. FRNK denotes the FAK-related non-kinase domain. Sites of tyrosine and serine phosphorylation are indicated. The amino-terminal PR domain is not shown. FAK binding partners are shown at their interaction sites within FAK. Binding of these proteins affects outcomes such as cell motility (dark blue), cell survival (light blue) or both functions (dark blue/light blue). Roles involving FAK activation are shown in grey, and important contributions to the tumour environment are shown in green. From [190] 40

Figure 17: The domain organization and activation of focal adhesion kinase (FAK). FAK is composed of a central kinase domain (KD), an amino-terminal side flanked by a protein band 4.1-ezrin-radixin-moesin (FERM) homology domain, and a carboxy-terminal focal adhesion targeting (FAT) domain flanked by proline-rich regions (PRRs). FAK localizes to focal adhesions and is triggered off by extracellular signals such as integrin-mediated adhesion and some growth factors. FAK is maintained in an inactive state by the binding of the FERM domain to the kinase domain, which blocks access to the catalytic site and sequesters the activation loop, as well as the key autophosphorylation site tyrosine 397 (Tyr397). Engagement of integrins with the extracellular matrix (ECM) or growth factors leads to signals that displace the

FERM domain, resulting in rapid autophosphorylation of Tyr397, which is a critical event in signal transduction by FAK. 41

Figure 18: Frequency of focal adhesion kinase (FAK) overexpression at protein level in human solid cancers. A Pubmed search of studies evaluating FAK protein expression in human cancers by immunohistochemistry (IHC) was performed to determine the percentage of cancer samples with increased FAK protein expression. 45

Figure 19: Frequency of increased focal adhesion kinase (FAK) expression at mRNA levels in human cancers. The Cancer Genome Atlas (TCGA) was queried using cbiportal.org to determine the percentage of tumor samples with increased levels of FAK mRNA expression. Search criteria included mRNA expression data (Z-scores for all genes) and tumor datasets with mRNA data. N = number of cancers analysed in the TCGA. 46

Figure 20: Frequency of focal adhesion kinase (FAK) genomic alterations in human cancers. The Cancer Genome Atlas (TCGA) was queried using cbiportal.org to determine the percentage of samples with FAK genomic alterations (mutations, fusions, amplifications, deep deletions, multiples alterations) in different cancers. Search criteria included PTK2 (FAK). N = number of cancers analysed in the TCGA. 47

Figure 21: FAK functions throughout the cell according to cellular localization (from [203].) FAK functions in both kinase-dependent and independent manners. Activated FAK goes to the nucleus and potentially regulates gene expression to affect cancer progression. Nuclear FAK functions as a scaffold for TP53 and MDM2 in a kinase-independent manner, increasing TP53 polyubiquitylation and degradation, thereby promoting cell survival[306,307]. 50

Figure 22: FAK and TP53 interactions at the cytoplasmic and nucleus compartments (from [306]). 51

Figure 23: Connexion between FAK and Rho-family GTPases at the focal adhesion complex (from [318]). 53

Figure 24: Pro-tumoral functions of FAK. (A). FAK is triggered off by integrins, G protein-coupled receptors (GPCR), growth factor receptors, and vascular endothelial growth factor receptor (VEGFR). Activated FAK promotes cell proliferation and survival. FAK also contributes to tumor progression and metastasis via cell adhesion, migration, and promotion of epithelial to mesenchymal transition (EMT). Transient contact between platelets and tumor cells induces TGFβ production by the platelets, which promotes EMT-like transformation and invasive behaviour. In endothelial cells (EC), FAK drives angiogenesis, increases vascular permeability, and regulates platelet extravasation; this facilitates intravasation or extravasation of tumor cells, leading to metastasis. Additionally, FAK induces a tumor protective fibrotic and immunosuppressive tumor microenvironment that promotes antitumor immune evasion. Indeed, FAK induces cytokines (short soluble (sST2), IL33, Ccl5), which lead

to the recruitment of immunosuppressive cells, such as regulatory T cells (Treg), tumor-associated macrophages (TAM), and GR1+ granulocytes, as well as to increased tumor fibrosis.	56
Figure 25: Ionizing radiations, chemotherapy, and reactive oxygen species (ROS) increase DNA damage and activate FAK in tumor cells. Activated FAK favors the expression of DNA damage repair (DDR) genes such as Growth Arrest and DNA Damage-inducible 45 (GADD45), Ataxia Telangiectasia Mutated (ATM) genes, and Ataxia Telangiectasia and Rad3-related (ATR) genes which play an important role in resistance to drug and radiation. Additionally, in endothelial cells (EC), ionizing radiations activate FAK and NF- κ B, which induces the production of various cytokines (IL-1 α , IL-2, IL-4 IL-6, IL-16) activating the proliferation of tumor cells. Abbreviations used in the figure and not described in the legend: IL-1RAcP: interleukin-1 receptor accessory protein, ST2L: longer membrane bound form.	56
Figure 26: RAC signaling pathways and effector functions. RAC1 cycle between an inactive GDP-bound form and an active GTP-bound form. Emphasis is given to pathways known to affect tumor related angiogenesis, cell survival, and metastasis (from [438]). Rho GTPases are also essential for the cytoskeletal changes underlying cell motility and invasion, which allow cancer cells to migrate away from the primary tumor and invade surrounding and later distant tissues, ultimately developing metastasis.	63
Figure 27: Association of focal adhesion kinase (FAK) amplification with survival. Kaplan-Meier overall survival, disease free and progression-free survival analysis of patients with versus without FAK amplification or mutation in their tumors (many different cancers included) in The Cancer Genome Atlas (TCGA) database (http://www.cbioportal.org/).	64
Figure 28: Tyramide signal amplification and multiplex immunostaining principle. Peroxidases, coupled to secondary antibodies, catalyze the deposition of several tyramide-fluorophore molecules at the site of the antigen. The covalent nature of tyramide-tyrosine bound allows for heat-mediated removal (stripping) of primary/secondary antibody pairs bound to the antigen, while preserving the antigen-associated fluorescence signal. This allows the sequential use of multiple primary antibodies of the same host species or isotype without the concern for crosstalk, thereby greatly enabling multiplexing potential on the same slide sample.	72
Figure 29: Fluorescent (p-FAK-FAK) and brightfield (pan-CK-hematoxylin) sequential image acquisition.	74
Figure 30: Fluorescent and brightfield images alignment.	74
Figure 31: Fluorescent (p-FAK-FAK) and brightfield (pan-CK-hematoxylin) scans merging and alignment.	75
Figure 32: Tumor delineation: detection based on pan-CK detection.	75

Figure 33: Illustration of FAK and phospho-FAK staining quantification on a tissue microarray section of non-small-cell lung cancer (NSCLC) stained by multiplex immunofluorescence (IF) immunohistochemistry (IHC). (A) TMA sections were sequentially stained by mIF with an antibody against phospho-FAK (red signal) and FAK (orange signal), followed by Hoechst nuclear marker (blue signal). After whole slide fluorescence image acquisitions, IHC was performed with a tumor marker using an antibody against pan-cytokeratin CKAE1-AE3 (CK, brown signal) on the same slide and digitalized with a slide scanner. (B) Each TMA plug was then automatically delineated via the image analysis tool Oncotopix version 2017.2 (Visiopharm). (C) CK-positive tumor regions were semi-automatically delineated from CK-negative stroma. (D) These tumor regions, detected on the brightfield scan, were transposed to the aligned fluorescent scan with Visiopharm Tissue Align module. (E) FAK and phospho-FAK stained areas were finally detected and quantified as illustrated for phospho-FAK in Fig. D.2., with staining detection according to three thresholds of intensity (low, yellow; medium, orange; high, red), while Fig. D.1. shows phospho-FAK staining without the mask. Original magnification: A, B, C: 1x; D: 2x; E: 20x. 76

Figure 34: Illustrations of FAK and phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence (IF) immunohistochemistry (IHC) in lung cancer and normal lung tissues. (A) Lung adenocarcinoma with absence of phospho-FAK expression but homogenous cytoplasmic FAK staining (orange) in tumor core, adjacent non-tumoral bronchi, and some stromal cells (including vessels and lymphoid structures). (B) Lung adenocarcinoma with nuclear phospho-FAK staining (red) and homogenous cytoplasmic FAK staining (orange). (C) Lung squamous carcinoma with absence of phospho-FAK expression but weak cytoplasmic FAK staining. (D) Lung squamous carcinoma with nuclear phospho-FAK staining (red) and homogenous cytoplasmic FAK staining (orange). (E) Small-cell lung cancer with nuclear phospho-FAK staining (red) and cytoplasmic FAK staining (orange). (F) Normal lung with cytoplasmic FAK staining in bronchi and some stromal cells (including vessels and lymphoid structures). (G) Lung squamous carcinoma used as negative control, showing the absence of phospho-FAK and FAK staining. Original magnification: 20x; scale bar: 50 μ m. 83

Figure 35: Quantification of FAK and phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence immunohistochemistry in 37 normal lung, 95 non-small-cell lung cancer (NSCLC), and 105 small-cell lung cancer (SCLC) tissues: (A) FAK staining score: percentage (%) of FAK-stained tumor area multiplied by (*) FAK mean intensity, (B) phospho-FAK (Y397) staining score: (% of phospho-FAK-stained tumor area of low intensity * 1) + (% of phospho-FAK-stained tumor area of medium intensity * 2) + (% of phospho-FAK-stained tumor area of high intensity * 3), and (C) ratio between phospho-FAK and FAK staining scores. Each dot represents one sample.

Data presented as the mean $\pm$ S.D. P-values were obtained using linear models and adjusted for multiple testing using the Bonferroni method.	84
Figure 36: Quantification of nuclear FAK and nuclear phospho-FAK (Y397) expression evaluated by multiplex immunofluorescence immunohistochemistry in 37 normal lung, 95 non-small-cell lung cancer (NSCLC), and 105 small-cell lung cancer (SCLC) tissues: (A) nuclear FAK staining score: percentage (%) of FAK-stained nucleus area multiplied by (*) nuclear FAK mean intensity, (B) nuclear phospho-FAK (Y397) staining score: (% of phospho-FAK-stained nucleus area of low intensity * 1) + (% of phospho-FAK-stained nucleus area of medium intensity * 2) + (% of phospho-FAK-stained nucleus area of high intensity * 3). Each dot represents one sample. Data presented as the mean $\pm$ S.D. P-values were obtained using linear models and adjusted for multiple testing using the Bonferroni method.	85
Figure 37: Quantification of (A) FAK and (B) phospho-FAK expression evaluated by Western blot (WB), with normalization to GAPDH expression, in nine normal lung, 30 non-small-cell lung cancer (NSCLC), and 10 small-cell lung cancer (SCLC) tissue lysates. Each dot represents one sample. Data presented as the mean $\pm$ S.D. Significance determined by Kruskal-Wallis test. (C) Illustration of a representative WB of FAK and phospho-FAK (Y397) expression in normal lung, NSCLC, and SCLC tissue lysates. All the WB are represented in Supplementary Fig. S1.	86
Figure 38: PF-573,228 (PF-228)'s effect on FAK expression/activity, cell proliferation, and cell cycle in SCLC cell lines.	100
Figure 39: PF-228's effect on apoptosis in SCLC cell lines.	102
Figure 40: PF-228's effect on migration and invasion in SCLC cell lines.	103
Figure 41: FAK Inhibitor 14 (Inh14) and PF-562,271 (PF-271)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H446 SCLC cell lines:	104
Figure 42: PF-573,228 (PF-228)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H82 SCLC cell lines transduced with FAK shRNA:	106
Figure 43: Effect of FAK shRNA and FAK-related non kinase (FRNK) transduction on FAK expression/ activity, cell proliferation, cell cycle, and apoptosis in SCLC cell lines.	108
Figure 44: Effect of FRNK transduction on FAK expression/activity, proliferation, apoptosis, and Rac1 expression in SCLC cell lines transduced with FAK shRNA. NCI-H446 cell lines were first stably transduced with FAK shRNA or no-target (NT) shRNA and then stably transduced with doxycycline-inducible FRNK-expression plasmid or pCLX empty vector as control. After transduction, they were submitted to puromycin and blasticidin-selection for two weeks. Finally, they were treated with doxycycline for 48h before the experiments.	110
Figure 45: phospho-Paxillin expression in NCI-H446 SCLC cell line where FAK has been inhibited with pharmacological and genetic methods:	113
Figure 46: A model of FAK as a regulator of Rac1 activation in SCLC.	118

Figure 47: Overall design of a potential translational research evaluating the safety and efficacy of a FAK TKI in a SCLC clinical trial, with associated preclinical studies, investigation of FAK & pFAK as a potential predictive biomarkers of FAK TKI efficacy as well as of FAK role in the mechanism of primary and acquired resistance to standard first and further-line therapies in SCLC.

123

8 TABLES

<i>Table 1: Clinical trials evaluating anti-angiogenic treatments in SCLC</i>	29
<i>Table 2: FAK inhibitors with anti-tumor activity in preclinical studies and clinical trials.</i>	43
<i>Table 3: Kinase selectivity profile of PF-228. The inhibitory effect of PF-228 on recombinant CDK1/cyclin B, CDK7/cyclin H, and GSK3 kinase activity was determined. The IC50 values for PF-228 on all kinases were 50–250 times greater than the IC50 for FAK. Adapted from [219].</i>	44
<i>Table 4: Clinical and pathological characteristics of NSCLC patients.</i>	79
<i>Table 5: Clinical and pathological characteristics of SCLC patients.</i>	80
<i>Table 6: Clinical and pathological characteristics of healthy donors.</i>	81
<i>Table 7: Correlation of FAK and phospho-FAK expression with recurrence-free survival and overall survival in (A) NSCLC and (B) SCLC patients in a univariate analysis.</i>	87
<i>Table 8: . Multivariate Cox proportional regression analysis for the association with recurrence-free survival and overall survival of (A) phospho-FAK staining score in NSCLC (n=95) and (B) the ratio between phospho-FAK and FAK staining scores in SCLC patients (n=105)</i>	88

9 LIST OF PUBLICATIONS, ABSTRACTS, ORAL PRESENTATIONS, GRANTS, AND HONORS RELATED TO THE THESIS WORK

9.1 Publications

- Ocak S, Friedman DB, Chen H, Ausborn JA, Hassanein M, Detry B, Weynand B, Aboubakar F, Pilette C, Sibille Y, Massion PP. Discovery of new membrane-associated proteins overexpressed in small-cell lung cancer. *Journal of Thoracic Oncology* 2014; 9(3):324-336
- Ladjemi MZ, Martin C, Lecocq M, Detry B, Aboubakar Nana F, Moulin C, et al. Increased IgA Expression in Lung Lymphoid Follicles in Severe COPD. *American journal of respiratory and critical care medicine* 2018
- Aboubakar Nana F, Lecocq M, Ladjemi MZ, Detry B, Dupasquier S, Feron O, et al. Therapeutic potential of Focal Adhesion Kinase inhibition in small cell lung cancer. *Molecular cancer therapeutics* 2018
- Aboubakar Nana, F.; Hoton, D.; Ambroise, J.; Lecocq, M.; Vanderputten, M.; Sibille, Y.; Vanaudenaerde, B.; Pilette, C.; Bouzin, C.; Ocak, S. Increased Expression and Activation of FAK in Small-Cell Lung Cancer Compared to Non-Small-Cell Lung Cancer. *Cancers* 2019, 11, 1526
- Aboubakar Nana, F.; Vanderputten, M.; Ocak, S. Role of Focal Adhesion Kinase in Small-Cell Lung Cancer and Its Potential as a Therapeutic Target. *Cancers* 2019, 11, 1683.

9.2 Abstracts

- Aboubakar F, Lecocq M, Maha L, Detry B, Massion PP, Sibille Y, Pilette C, Ocak S. Inhibition of the Focal Adhesion Kinase has anti-tumoral effect in small-cell lung cancer cell lines. *Belgian Society for Pneumology (BVP-SBP), GSK Clinical and Basic Science Award*; June 11, 2014; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Detry B, Massion PP, Sibille Y, Pilette C, Ocak S. Inhibition of the Focal Adhesion Kinase has anti-tumoral effect in small-cell lung cancer cell lines. *Institut de Recherche Expérimentale et Clinique (IREC) Ph.D. Day*; September 26, 2014; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Detry B, Massion PP, Sibille Y, Pilette C, Ocak S. Inhibition of the focal adhesion kinase has anti-tumoral effect in small-cell lung cancer cell lines. *5th European Lung Cancer Conference (ELCC)*; April 15-18, 2015; Geneva, Switzerland
- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. FAK inhibition by PF228 has anti-tumoral effects associated with inhibition of Histone 3 and Aurora Kinases A/B phosphorylation in SCLC. *16th International Association for the Study of Lung Cancer (IASLC) World Conference*; September 7, 2015; Denver, USA

- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. Focal Adhesion Kinase inhibition has antitumoral effects in small-cell lung cancer. IREC 3rd PhD Day; March 4, 2016; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. Dualité du rôle de la kinase d'adhésion focale dans les lignées cellulaires humaines de cancer bronchique à petites cellules. 12^{ème} Journée de recherche Respiratoire ; October 4, 2016 ; Nice, France
- Aboubakar F, Dieu M, Fransolet M, Lecocq M, Sibille Y, Pilette C, Michiels C, Ocak S. High-throughput phospho-proteomic analysis of a small-cell lung cancer cell line treated with a Focal Adhesion Kinase inhibitor; NARILIS research day; November 17, 2016; Namur, Belgium
- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. Dual Role of the Focal Adhesion Kinase in Small-Cell Lung Cancer. 17th International Association for the Study of Lung Cancer (IASLC) World Conference; December 4, 2016; Vienna, Austria
- Aboubakar F, Lecocq M, Maha L, Detry B, Dupasquier S, Massion PP, Feron O, Sibille Y, Pilette C, Ocak S. Targeting Focal Adhesion Kinase in small-cell lung cancer. TELEVIE's Researchers Seminar; December 8, 2016; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. Distinct role of FAK kinase and C-terminal domains on small-cell lung cancer proliferation. 18th International Association for the Study of Lung Cancer (IASLC) World Conference; October 8, 2017; Yokohama, Japan

9.3 Peer-reviewed oral presentations

- Aboubakar F, Lecocq M, Maha L, Detry B, Massion PP, Sibille Y, Pilette C, Ocak S. Inhibition of the Focal Adhesion Kinase has anti-tumoral effect in small-cell lung cancer cell lines. Belgian Society for Pneumology (BVP-SBP), GSK Clinical and Basic Science Award; June 11, 2014; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Detry B, Massion PP, Sibille Y, Pilette C, Ocak S. Inhibition of the Focal Adhesion Kinase has anti-tumoral effect in small-cell lung cancer cell lines. IREC Ph.D. Day; September 26, 2014; Brussels, Belgium
- Aboubakar F, Lecocq M, Maha L, Dupasquier S, Detry B, Massion PP, Pilette C, Sibille Y, Ocak S. Dualité du rôle de la kinase d'adhésion focale dans les lignées cellulaires humaines de cancer bronchique à petites cellules. 12^{ème} Journée de recherche Respiratoire ; October 4, 2016 ; Nice, France

9.4 Grants

- 2013-2015: Bourse du Fonds Scientifique pour la Recherche (FSR), belgique; "Role of Focal Adhesion Kinase (FAK) in the invasive phenotype

of small-cell lung cancer (SCLC) and analysis of FAK signaling in SCLC by quantitative phosphoproteomics" (75.000€)

- 2015-2017: Bourse du Télévie, Belgique; "Antitumoral potential of targeting the Focal Adhesion Kinase activity in small-cell lung cancer" (full-time PhD student salary and 7.000€)
- 2015-2016: Bourse de la Fondation Willy & Marcy De Vooght, Belgique; «Antitumoral potential of targeting the Focal Adhesion Kinase in small-cell lung cancer» (12.000€)
- 2017-2018: Bourse de la Fondation Saint-Luc, Belgique ; « Characterization of the Focal Adhesion Pathway in chemoresistance of small cell lung cancer» (30.000€)
- 2018-2019: FSR Grant; "Focal Adhesion Kinase activation in SCLC: mechanisms, prognostic implication, and therapeutic potential of its inhibition" (66.000 €)
- 2019-2020: Grant FRC (Fond de recherche Clinique)

10 REFERENCES

1. (IARC), G. GLOBOCAN 2018 (IARC) , Section of Cancer Surveillance. Accessed January 6, 2019.
2. Pirie, K.; Peto, R.; Reeves, G.K.; Green, J.; Beral, V. The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK. *The Lancet* **2013**, *381*, 133-141, doi:10.1016/S0140-6736(12)61720-6.
3. Jha, P.; Ramasundarahettige, C.; Landsman, V.; Rostron, B.; Thun, M.; Anderson, R.N.; McAfee, T.; Peto, R. 21st-Century Hazards of Smoking and Benefits of Cessation in the United States. *New England Journal of Medicine* **2013**, *368*, 341-350, doi:10.1056/NEJMSa1211128.
4. Jha, P.; Peto, R. Global Effects of Smoking, of Quitting, and of Taxing Tobacco. *New England Journal of Medicine* **2014**, *370*, 60-68, doi:10.1056/NEJMra1308383.
5. Ordóñez-Mena, J.M.; Schöttker, B.; Mons, U.; Jenab, M.; Freisling, H.; Bueno-de-Mesquita, B.; O'Doherty, M.G.; Scott, A.; Kee, F.; Stricker, B.H., et al. Quantification of the smoking-associated cancer risk with rate advancement periods: meta-analysis of individual participant data from cohorts of the CHANCES consortium. *BMC Medicine* **2016**, *14*, 62, doi:10.1186/s12916-016-0607-5.
6. Malhotra, J.; Borron, C.; Freedman, N.D.; Abnet, C.C.; van den Brandt, P.A.; White, E.; Milne, R.L.; Giles, G.G.; Boffetta, P. Association between Cigar or Pipe Smoking and Cancer Risk in Men: A Pooled Analysis of Five Cohort Studies. *Cancer prevention research (Philadelphia, Pa.)* **2017**, *10*, 704-709, doi:10.1158/1940-6207.capr-17-0084.
7. Pleasance, E.D.; Stephens, P.J.; O'Meara, S.; McBride, D.J.; Meynert, A.; Jones, D.; Lin, M.L.; Beare, D.; Lau, K.W.; Greenman, C., et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature* **2010**, *463*, 184-190, doi:10.1038/nature08629.
8. Govindan, R.; Ding, L.; Griffith, M.; Subramanian, J.; Dees, Nathan D.; Kanchi, Krishna L.; Maher, Christopher A.; Fulton, R.; Fulton, L.; Wallis, J., et al. Genomic Landscape of Non-Small Cell Lung Cancer in Smokers and Never-Smokers. *Cell* **150**, 1121-1134, doi:10.1016/j.cell.2012.08.024.
9. Pfeifer, G.P.; Denissenko, M.F.; Olivier, M.; Tretyakova, N.; Hecht, S.S.; Hainaut, P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* **2002**, *21*, 7435-7451, doi:10.1038/sj.onc.1205803.
10. Darby, S.; Hill, D.; Deo, H.; Auvinen, A.; Barros-Dios, J.M.; Baysson, H.; Bochicchio, F.; Falk, R.; Farchi, S.; Figueiras, A., et al. Residential radon and lung cancer—detailed results of a collaborative analysis of individual data on 7148

persons with lung cancer and 14 208 persons without lung cancer from 13 epidemiologic studies in Europe. *Scandinavian Journal of Work, Environment & Health* **2006**, 1-84.

11. Frost, G.; Darnton, A.; Harding, A.H. The effect of smoking on the risk of lung cancer mortality for asbestos workers in Great Britain (1971-2005). *The Annals of occupational hygiene* **2011**, 55, 239-247, doi:10.1093/annhyg/meq089.

12. Raaschou-Nielsen, O.; Andersen, Z.J.; Beelen, R.; Samoli, E.; Stafoggia, M.; Weinmayr, G.; Hoffmann, B.; Fischer, P.; Nieuwenhuijsen, M.J.; Brunekreef, B., et al. Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE). *The Lancet Oncology* **2013**, 14, 813-822, doi:10.1016/S1470-2045(13)70279-1.

13. Olsson, A.C.; Vermeulen, R.; Schüz, J.; Kromhout, H.; Pesch, B.; Peters, S.; Behrens, T.; Portengen, L.; Mirabelli, D.; Gustavsson, P., et al. Exposure-Response Analyses of Asbestos and Lung Cancer Subtypes in a Pooled Analysis of Case-Control Studies. *Epidemiology (Cambridge, Mass.)* **2017**, 28, 288-299, doi:10.1097/EDE.0000000000000604.

14. Rodriguez-Martinez, A.; Torres-Duran, M.; Barros-Dios, J.M.; Ruano-Ravina, A. Residential radon and small cell lung cancer. A systematic review. *Cancer letters* **2018**, 426, 57-62, doi:10.1016/j.canlet.2018.04.003.

15. Toh, C.K.; Gao, F.; Lim, W.T.; Leong, S.S.; Fong, K.W.; Yap, S.P.; Hsu, A.A.; Eng, P.; Koong, H.N.; Thirugnanam, A., et al. Never-smokers with lung cancer: epidemiologic evidence of a distinct disease entity. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2006**, 24, 2245-2251, doi:10.1200/jco.2005.04.8033.

16. Foeglé, J.; Hédelin, G.; Lebitasy, M.-P.; Purohit, A.; Velten, M.; Quoix, E. Specific Features of Non-small Cell Lung Cancer in Women: A Retrospective Study of 1738 Cases Diagnosed in Bas-Rhin between 1982 and 1997. *Journal of Thoracic Oncology* **2007**, 2, 466-474, doi:<https://doi.org/10.1097/01.JTO.0000275340.39960.25>.

17. Sun, S.; Schiller, J.H.; Gazdar, A.F. Lung cancer in never smokers--a different disease. *Nature reviews. Cancer* **2007**, 7, 778-790, doi:10.1038/nrc2190.

18. Houston, K.A.; Henley, S.J.; Li, J.; White, M.C.; Richards, T.B. Patterns in lung cancer incidence rates and trends by histologic type in the United States, 2004-2009. *Lung cancer* **2014**, 86, 22-28, doi:10.1016/j.lungcan.2014.08.001.

19. Succony, L.; Janes, S.M. Airway stem cells and lung cancer. *QJM : monthly journal of the Association of Physicians* **2014**, 107, 607-612, doi:10.1093/qjmed/hcu040.

20. Govindan, R.; Page, N.; Morgensztern, D.; Read, W.; Tierney, R.; Vlahiotis, A.; Spitznagel, E.L.; Piccirillo, J. Changing Epidemiology of Small-Cell Lung Cancer in the United States Over the Last 30 Years: Analysis of the Surveillance,

Epidemiologic, and End Results Database. *Journal of Clinical Oncology* **2006**, *24*, 4539-4544, doi:10.1200/jco.2005.04.4859.

21. Behera, M.; Ragin, C.; Kim, S.; Pillai, R.N.; Chen, Z.; Steuer, C.E.; Saba, N.F.; Belani, C.P.; Khuri, F.R.; Ramalingam, S.S., et al. Trends, predictors, and impact of systemic chemotherapy in small cell lung cancer patients between 1985 and 2005. *Cancer* **2015**, 10.1002/cncr.29674, doi:10.1002/cncr.29674.

22. Group, N.M.-A.C. Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2008**, *26*, 4617-4625, doi:10.1200/JCO.2008.17.7162.

23. Barlesi, F.; Mazieres, J.; Merlio, J.P.; Debieuvre, D.; Mosser, J.; Lena, H.; Ouafik, L.; Besse, B.; Rouquette, I.; Westeel, V., et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). *Lancet (London, England)* **2016**, *387*, 1415-1426, doi:10.1016/s0140-6736(16)00004-0.

24. Couraud, S.; Souquet, P.J.; Paris, C.; Do, P.; Doubre, H.; Pichon, E.; Dixmier, A.; Monnet, I.; Etienne-Mastroianni, B.; Vincent, M., et al. BioCAST/IFCT-1002: epidemiological and molecular features of lung cancer in never-smokers. *The European respiratory journal* **2015**, *45*, 1403-1414, doi:10.1183/09031936.00097214.

25. Morgensztern, D.; Ng, S.H.; Gao, F.; Govindan, R. Trends in Stage Distribution for Patients with Non-small Cell Lung Cancer: A National Cancer Database Survey. *Journal of Thoracic Oncology* **2010**, *5*, 29-33, doi:<http://dx.doi.org/10.1097/JTO.0b013e3181c5920c>.

26. Goldstraw, P.; Chansky, K.; Crowley, J.; Rami-Porta, R.; Asamura, H.; Eberhardt, W.E.E.; Nicholson, A.G.; Groome, P.; Mitchell, A.; Bolejack, V., et al. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. *Journal of Thoracic Oncology* **2016**, *11*, 39-51, doi:<https://doi.org/10.1016/j.jtho.2015.09.009>.

27. Detterbeck, F.C.; Boffa, D.J.; Kim, A.W.; Tanoue, L.T. The Eighth Edition Lung Cancer Stage Classification. *CHEST* **2017**, *151*, 193-203, doi:10.1016/j.chest.2016.10.010.

28. Antonia, S.J.; Villegas, A.; Daniel, D.; Vicente, D.; Murakami, S.; Hui, R.; Kurata, T.; Chiappori, A.; Lee, K.H.; de Wit, M., et al. Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. *New England Journal of Medicine* *0*, null, doi:10.1056/NEJMoa1809697.

29. Garon, E.B.; Hellmann, M.D.; Rizvi, N.A.; Carcereny, E.; Leighl, N.B.; Ahn, M.-J.; Eder, J.P.; Balmanoukian, A.S.; Aggarwal, C.; Horn, L., et al. Five-Year Overall Survival for Patients With Advanced Non–Small-Cell Lung Cancer Treated With

Pembrolizumab: Results From the Phase I KEYNOTE-001 Study. *Journal of Clinical Oncology* 0, JCO.19.00934, doi:10.1200/jco.19.00934.

30. Planchard, D.; Popat, S.; Kerr, K.; Novello, S.; Smit, E.F.; Faivre-Finn, C.; Mok, T.S.; Reck, M.; Van Schil, P.E.; Hellmann, M.D., et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology* **2018**, 29, iv192-iv237, doi:10.1093/annonc/mdy275.

31. Martinez, P.; Peters, S.; Stammers, T.; Soria, J.-C. Immunotherapy for the First-Line Treatment of Patients with Metastatic Non-Small Cell Lung Cancer. *Clinical Cancer Research* **2019**, 25, 2691-2698, doi:10.1158/1078-0432.Ccr-18-3904.

32. Rosell, R.; Moran, T.; Queralt, C.; Porta, R.; Cardenal, F.; Camps, C.; Majem, M.; Lopez-Vivanco, G.; Isla, D.; Provencio, M., et al. Screening for epidermal growth factor receptor mutations in lung cancer. *The New England journal of medicine* **2009**, 361, 958-967, doi:10.1056/NEJMoa0904554.

33. Dearden, S.; Stevens, J.; Wu, Y.L.; Blowers, D. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Annals of oncology : official journal of the European Society for Medical Oncology* **2013**, 24, 2371-2376, doi:10.1093/annonc/mdt205.

34. Bergethon, K.; Shaw, A.T.; Ou, S.H.; Katayama, R.; Lovly, C.M.; McDonald, N.T.; Massion, P.P.; Siwak-Tapp, C.; Gonzalez, A.; Fang, R., et al. ROS1 rearrangements define a unique molecular class of lung cancers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2012**, 30, 863-870, doi:10.1200/jco.2011.35.6345.

35. Shi, Y.; Au, J.S.-K.; Thongprasert, S.; Srinivasan, S.; Tsai, C.-M.; Khoa, M.T.; Heeroma, K.; Itoh, Y.; Cornelio, G.; Yang, P.-C. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* **2014**, 9, 154-162, doi:10.1097/JTO.000000000000033.

36. Dong, H.; Strome, S.E.; Salomao, D.R.; Tamura, H.; Hirano, F.; Flies, D.B.; Roche, P.C.; Lu, J.; Zhu, G.; Tamada, K., et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature medicine* **2002**, 8, 793, doi:10.1038/nm730

<https://www.nature.com/articles/nm730#supplementary-information>.

37. Leach, D.R.; Krummel, M.F.; Allison, J.P. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* **1996**, 271, 1734-1736.

38. Herbst, R.S.; Baas, P.; Kim, D.-W.; Felip, E.; Pérez-Gracia, J.L.; Han, J.-Y.; Molina, J.; Kim, J.-H.; Arvis, C.D.; Ahn, M.-J., et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *The Lancet* **387**, 1540-1550, doi:10.1016/S0140-6736(15)01281-7.

39. Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D.R.; Steins, M.; Ready, N.E.; Chow, L.Q.; Vokes, E.E.; Felip, E.; Holgado, E., et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *The New England journal of medicine* **2015**, 373, 1627-1639, doi:10.1056/NEJMoa1507643.
40. Brahmer, J.; Reckamp, K.L.; Baas, P.; Crino, L.; Eberhardt, W.E.; Poddubskaya, E.; Antonia, S.; Pluzanski, A.; Vokes, E.E.; Holgado, E., et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *The New England journal of medicine* **2015**, 373, 123-135, doi:10.1056/NEJMoa1504627.
41. Rittmeyer, A.; Barlesi, F.; Waterkamp, D.; Park, K.; Ciardiello, F.; von Pawel, J.; Gadgeel, S.M.; Hida, T.; Kowalski, D.M.; Dols, M.C., et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet (London, England)* **2017**, 389, 255-265, doi:10.1016/s0140-6736(16)32517-x.
42. Reck, M.; Rodríguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csőszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S., et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. *New England Journal of Medicine* **2016**, 375, 1823-1833, doi:10.1056/NEJMoa1606774.
43. Paz-Ares, L.; Luft, A.; Vicente, D.; Tafreshi, A.; Gümüş, M.; Mazières, J.; Hermes, B.; Çay Şenler, F.; Csőszi, T.; Fülöp, A., et al. Pembrolizumab plus Chemotherapy for Squamous Non–Small-Cell Lung Cancer. *New England Journal of Medicine* **2018**, 379, 2040-2051, doi:10.1056/NEJMoa1810865.
44. Socinski, M.A.; Jotte, R.M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodríguez-Abreu, D.; Moro-Sibilot, D.; Thomas, C.A.; Barlesi, F., et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *New England Journal of Medicine* **2018**, 378, 2288-2301, doi:10.1056/NEJMoa1716948.
45. Gaspar, L.E.; McNamara, E.J.; Gay, E.G.; Putnam, J.B.; Crawford, J.; Herbst, R.S.; Bonner, J.A. Small-Cell Lung Cancer: Prognostic Factors and Changing Treatment Over 15 Years. *Clinical Lung Cancer* **2012**, 13, 115-122, doi:<https://doi.org/10.1016/j.clcc.2011.05.008>.
46. Yu, J.B.; Decker, R.H.; Detterbeck, F.C.; Wilson, L.D. Surveillance epidemiology and end results evaluation of the role of surgery for stage I small cell lung cancer. *J Thorac Oncol* **2010**, 5, 215-219, doi:10.1097/JTO.0b013e3181cd3208.
47. Schreiber, D.; Rineer, J.; Weedon, J.; Vongtama, D.; Wortham, A.; Kim, A.; Han, P.; Choi, K.; Rotman, M. Survival outcomes with the use of surgery in limited-stage small cell lung cancer: should its role be re-evaluated? *Cancer* **2010**, 116, 1350-1357, doi:10.1002/cncr.24853.
48. Früh, M.; De Ruyscher, D.; Popat, S.; Crinò, L.; Peters, S.; Felip, E.; on behalf of the, E.G.W.G. Small-cell lung cancer (SCLC): ESMO Clinical Practice

Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology* **2013**, *24*, vi99-vi105, doi:10.1093/annonc/mdt178.

49. Kubota, K.; Hida, T.; Ishikura, S.; Mizusawa, J.; Nishio, M.; Kawahara, M.; Yokoyama, A.; Imamura, F.; Takeda, K.; Negoro, S., et al. Etoposide and cisplatin versus irinotecan and cisplatin in patients with limited-stage small-cell lung cancer treated with etoposide and cisplatin plus concurrent accelerated hyperfractionated thoracic radiotherapy (JCOG0202): a randomised phase 3 study. *The Lancet Oncology* **2014**, *15*, 106-113, doi:10.1016/S1470-2045(13)70511-4.

50. Turrisi, A.T., 3rd; Kim, K.; Blum, R.; Sause, W.T.; Livingston, R.B.; Komaki, R.; Wagner, H.; Aisner, S.; Johnson, D.H. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med* **1999**, *340*, 265-271, doi:10.1056/NEJM199901283400403.

51. Pignon, J.-P.; Arriagada, R.; Ihde, D.C.; Johnson, D.H.; Perry, M.C.; Souhami, R.L.; Brodin, O.; Joss, R.A.; Kies, M.S.; Lebeau, B., et al. A Meta-Analysis of Thoracic Radiotherapy for Small-Cell Lung Cancer. *New England Journal of Medicine* **1992**, *327*, 1618-1624, doi:10.1056/nejm199212033272302.

52. De Ruyscher, D.; Pijls-Johannesma, M.; Bentzen, S.M.; Minken, A.; Wanders, R.; Lutgens, L.; Hochstenbag, M.; Boersma, L.; Wouters, B.; Lammering, G., et al. Time between the first day of chemotherapy and the last day of chest radiation is the most important predictor of survival in limited-disease small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2006**, *24*, 1057-1063, doi:10.1200/jco.2005.02.9793.

53. De Ruyscher, D.; Pijls-Johannesma, M.; Vansteenkiste, J.; Kester, A.; Rutten, I.; Lambin, P. Systematic review and meta-analysis of randomised, controlled trials of the timing of chest radiotherapy in patients with limited-stage, small-cell lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2006**, *17*, 543-552, doi:10.1093/annonc/mdj094.

54. Sabari, J.K.; Lok, B.H.; Laird, J.H.; Poirier, J.T.; Rudin, C.M. Unravelling the biology of SCLC: implications for therapy. *Nature reviews. Clinical oncology* **2017**, *14*, 549-561, doi:10.1038/nrclinonc.2017.71.

55. Pujol, J.L.; Daures, J.P.; Riviere, A.; Quoix, E.; Westeel, V.; Quantin, X.; Breton, J.L.; Lemarie, E.; Poudenx, M.; Milleron, B., et al. Etoposide plus cisplatin with or without the combination of 4'-epidoxorubicin plus cyclophosphamide in treatment of extensive small-cell lung cancer: a French Federation of Cancer Institutes multicenter phase III randomized study. *Journal of the National Cancer Institute* **2001**, *93*, 300-308.

56. Pujol, J.L.; Carestia, L.; Daures, J.P. Is there a case for cisplatin in the treatment of small-cell lung cancer? A meta-analysis of randomized trials of a cisplatin-containing regimen versus a regimen without this alkylating agent. *British journal of cancer* **2000**, *83*, 8-15, doi:10.1054/bjoc.2000.1164.

57. van Meerbeeck, J.P.; Fennell, D.A.; De Ruysscher, D.K.M. Small-cell lung cancer. *The Lancet* **378**, 1741-1755, doi:[http://dx.doi.org/10.1016/S0140-6736\(11\)60165-7](http://dx.doi.org/10.1016/S0140-6736(11)60165-7).
58. Reck, M.; Luft, A.; Szczesna, A.; Havel, L.; Kim, S.-W.; Akerley, W.; Pietanza, M.C.; Wu, Y.-I.; Zielinski, C.; Thomas, M., et al. Phase III Randomized Trial of Ipilimumab Plus Etoposide and Platinum Versus Placebo Plus Etoposide and Platinum in Extensive-Stage Small-Cell Lung Cancer. *Journal of Clinical Oncology* **2016**, *34*, 3740-3748, doi:10.1200/jco.2016.67.6601.
59. Squibb, B.-M. CheckMate -451 Study Did Not Meet Primary Endpoint of Overall Survival with Opdivo Plus Yervoy Vs. Placebo as A Maintenance Therapy in Patients with Extensive-Stage Small Cell Lung Cancer After Completion of First-Line. *Bristol-Myers Squibb, Accessed January 4, 2019 Published November 26, 2018*.
60. Antonia, S.J.; Lopez-Martin, J.A.; Bendell, J.; Ott, P.A.; Taylor, M.; Eder, J.P.; Jager, D.; Pietanza, M.C.; Le, D.T.; de Braud, F., et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. *The Lancet. Oncology* **2016**, *17*, 883-895, doi:10.1016/s1470-2045(16)30098-5.
61. Rudin, C.M.; Pietanza, M.C.; Bauer, T.M.; Ready, N.; Morgensztern, D.; Glisson, B.S.; Byers, L.A.; Johnson, M.L.; Burris, H.A., 3rd; Robert, F., et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. *The Lancet. Oncology* **2017**, *18*, 42-51, doi:10.1016/s1470-2045(16)30565-4.
62. Horn, L.; Mansfield, A.S.; Szcześna, A.; Havel, L.; Krzakowski, M.; Hochmair, M.J.; Huemer, F.; Losonczy, G.; Johnson, M.L.; Nishio, M., et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. *New England Journal of Medicine* **2018**, *379*, 2220-2229, doi:10.1056/NEJMoa1809064.
63. Paz-Ares, L.; Dvorkin, M.; Chen, Y.; Reinmuth, N.; Hotta, K.; Trukhin, D.; Statsenko, G.; Hochmair, M.J.; Özgüroğlu, M.; Ji, J.H., et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. *Lancet (London, England)* **2019**, *394*, 1929-1939, doi:10.1016/s0140-6736(19)32222-6.
64. Owonikoko, T.K.; Behera, M.; Chen, Z.; Bhimani, C.; Curran, W.J.; Khuri, F.R.; Ramalingam, S.S. A systematic analysis of efficacy of second-line chemotherapy in sensitive and refractory small-cell lung cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* **2012**, *7*, 866-872, doi:10.1097/JTO.0b013e31824c7f4b.
65. Goto, K.; Ohe, Y.; Shibata, T.; Seto, T.; Takahashi, T.; Nakagawa, K.; Tanaka, H.; Takeda, K.; Nishio, M.; Mori, K., et al. Combined chemotherapy with cisplatin, etoposide, and irinotecan versus topotecan alone as second-line treatment

for patients with sensitive relapsed small-cell lung cancer (JCOG0605): a multicentre, open-label, randomised phase 3 trial. *The Lancet. Oncology* **2016**, *17*, 1147-1157, doi:10.1016/s1470-2045(16)30104-8.

66. N. Baize, I.M., L. Greillier, M. Geier, H. Lena, H. Janicot, A. Vergnenegre, J. Crequit, R. Lamy, J.B. Auliac, J. Le Treut, H. Le Caer, R. Gervais, E. Dansin, A. Madroszyk, P. Renault, G. Legarff, R. Schott, P. Saulnier, C. Chouaid. Carboplatin-etoposide versus topotecan as second-line treatment for sensitive relapsed small-cell lung cancer: Phase 3 trial. *J Thorac Oncol* **2019**;14(suppl):Abstr OA15.02 **IASLC 2019 World Conference on Lung Cancer**.

67. O'Brien, M.E.; Ciuleanu, T.E.; Tsekov, H.; Shparyk, Y.; Cuceviá, B.; Juhasz, G.; Thatcher, N.; Ross, G.A.; Dane, G.C.; Crofts, T. Phase III trial comparing supportive care alone with supportive care with oral topotecan in patients with relapsed small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2006**, *24*, 5441-5447, doi:10.1200/jco.2006.06.5821.

68. Eckardt, J.R.; von Pawel, J.; Pujol, J.L.; Papai, Z.; Quoix, E.; Ardizzoni, A.; Poulin, R.; Preston, A.J.; Dane, G.; Ross, G. Phase III study of oral compared with intravenous topotecan as second-line therapy in small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2007**, *25*, 2086-2092, doi:10.1200/jco.2006.08.3998.

69. Ardizzoni, A.; Hansen, H.; Dombrowsky, P.; Gamucci, T.; Kaplan, S.; Postmus, P.; Giaccone, G.; Schaefer, B.; Wanders, J.; Verweij, J. Topotecan, a new active drug in the second-line treatment of small-cell lung cancer: a phase II study in patients with refractory and sensitive disease. The European Organization for Research and Treatment of Cancer Early Clinical Studies Group and New Drug Development Office, and the Lung Cancer Cooperative Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1997**, *15*, 2090-2096, doi:10.1200/jco.1997.15.5.2090.

70. von Pawel, J.; Schiller, J.H.; Shepherd, F.A.; Fields, S.Z.; Kleisbauer, J.P.; Chrysson, N.G.; Stewart, D.J.; Clark, P.I.; Palmer, M.C.; Depierre, A., et al. Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1999**, *17*, 658-667, doi:10.1200/jco.1999.17.2.658.

71. Masuda, N.; Fukuoka, M.; Kusunoki, Y.; Matsui, K.; Takifuji, N.; Kudoh, S.; Negoro, S.; Nishioka, M.; Nakagawa, K.; Takada, M. CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1992**, *10*, 1225-1229, doi:10.1200/jco.1992.10.8.1225.

72. Smit, E.F.; Fokkema, E.; Biesma, B.; Groen, H.J.; Snoek, W.; Postmus, P.E. A phase II study of paclitaxel in heavily pretreated patients with small-cell lung cancer. *British journal of cancer* **1998**, *77*, 347-351, doi:10.1038/bjc.1998.54.

73. Yamamoto, N.; Tsurutani, J.; Yoshimura, N.; Asai, G.; Moriyama, A.; Nakagawa, K.; Kudoh, S.; Takada, M.; Minato, Y.; Fukuoka, M. Phase II study of weekly paclitaxel for relapsed and refractory small cell lung cancer. *Anticancer research* **2006**, 26, 777-781.
74. Smyth, J.F.; Smith, I.E.; Sessa, C.; Schoffski, P.; Wanders, J.; Franklin, H.; Kaye, S.B. Activity of docetaxel (Taxotere) in small cell lung cancer. The Early Clinical Trials Group of the EORTC. *Eur J Cancer* **1994**, 30a, 1058-1060, doi:10.1016/0959-8049(94)90455-3.
75. van der Lee, I.; Smit, E.F.; van Putten, J.W.; Groen, H.J.; Schlösser, N.J.; Postmus, P.E.; Schramel, F.M. Single-agent gemcitabine in patients with resistant small-cell lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2001**, 12, 557-561, doi:10.1023/a:1011104509759.
76. Masters, G.A.; Declerck, L.; Blanke, C.; Sandler, A.; DeVore, R.; Miller, K.; Johnson, D. Phase II trial of gemcitabine in refractory or relapsed small-cell lung cancer: Eastern Cooperative Oncology Group Trial 1597. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2003**, 21, 1550-1555, doi:10.1200/jco.2003.09.130.
77. Jassem, J.; Karnicka-Młodkowska, H.; van Pottelsberghe, C.; van Glabbeke, M.; Nosedà, M.A.; Ardizzoni, A.; Gozzelino, F.; Planting, A.; van Zandwijk, N. Phase II study of vinorelbine (Navelbine) in previously treated small cell lung cancer patients. EORTC Lung Cancer Cooperative Group. *Eur J Cancer* **1993**, 29a, 1720-1722, doi:10.1016/0959-8049(93)90112-s.
78. Furuse, K.; Kubota, K.; Kawahara, M.; Takada, M.; Kimura, I.; Fujii, M.; Ohta, M.; Hasegawa, K.; Yoshida, K.; Nakajima, S., et al. Phase II study of vinorelbine in heavily previously treated small cell lung cancer. Japan Lung Cancer Vinorelbine Study Group. *Oncology* **1996**, 53, 169-172, doi:10.1159/000227555.
79. Pietanza, M.C.; Kadota, K.; Huberman, K.; Sima, C.S.; Fiore, J.J.; Sumner, D.K.; Travis, W.D.; Heguy, A.; Ginsberg, M.S.; Holodny, A.I., et al. Phase II trial of temozolomide in patients with relapsed sensitive or refractory small cell lung cancer, with assessment of methylguanine-DNA methyltransferase as a potential biomarker. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2012**, 18, 1138-1145, doi:10.1158/1078-0432.Ccr-11-2059.
80. Zauderer, M.G.; Drilon, A.; Kadota, K.; Huberman, K.; Sima, C.S.; Bergagnini, I.; Sumner, D.K.; Travis, W.D.; Heguy, A.; Ginsberg, M.S., et al. Trial of a 5-day dosing regimen of temozolomide in patients with relapsed small cell lung cancers with assessment of methylguanine-DNA methyltransferase. *Lung cancer* **2014**, 86, 237-240, doi:10.1016/j.lungcan.2014.08.007.
81. Johnson, D.H.; Greco, F.A.; Strupp, J.; Hande, K.R.; Hainsworth, J.D. Prolonged administration of oral etoposide in patients with relapsed or refractory small-cell lung cancer: a phase II trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1990**, 8, 1613-1617, doi:10.1200/jco.1990.8.10.1613.

82. Einhorn, L.H.; Pennington, K.; McClean, J. Phase II trial of daily oral VP-16 in refractory small cell lung cancer: a Hoosier Oncology Group study. *Semin Oncol* **1990**, *17*, 32-35.
83. Trigo, J.; Subbiah, V.; Besse, B.; Moreno, V.; López, R.; Sala, M.A.; Peters, S.; Ponce, S.; Fernández, C.; Alfaro, V., et al. Lurbinectedin as second-line treatment for patients with small-cell lung cancer: a single-arm, open-label, phase 2 basket trial. *The Lancet. Oncology* **2020**, *21*, 645-654, doi:10.1016/s1470-2045(20)30068-1.
84. Lammers, P.E.; Shyr, Y.; Li, C.I.; Hutchison, A.S.; Sandler, A.; Carbone, D.P.; Johnson, D.H.; Keedy, V.L.; Horn, L. Phase II study of bendamustine in relapsed chemotherapy sensitive or resistant small-cell lung cancer. *J Thorac Oncol* **2014**, *9*, 559-562, doi:10.1097/jto.0000000000000079.
85. Hellmann, M.D.; Callahan, M.K.; Awad, M.M.; Calvo, E.; Ascierto, P.A.; Atmaca, A.; Rizvi, N.A.; Hirsch, F.R.; Selvaggi, G.; Szustakowski, J.D., et al. Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in Combination with Ipilimumab in Small-Cell Lung Cancer. *Cancer cell* **2018**, *33*, 853-861.e854, doi:10.1016/j.ccell.2018.04.001.
86. Cancer, N.g.V.S.C.L. **Accessed May 21, 2020.**
87. Chung, H.C.; Piha-Paul, S.A.; Lopez-Martin, J.; Schellens, J.H.M.; Kao, S.; Miller, W.H., Jr.; Delord, J.-P.; Gao, B.; Planchard, D.; Gottfried, M., et al. Pembrolizumab After Two or More Lines of Previous Therapy in Patients With Recurrent or Metastatic SCLC: Results From the KEYNOTE-028 and KEYNOTE-158 Studies. *Journal of Thoracic Oncology* **2020**, *15*, 618-627, doi:10.1016/j.jtho.2019.12.109.
88. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: the next generation. *Cell* **2011**, *144*, 646-674, doi:10.1016/j.cell.2011.02.013.
89. Hicklin, D.J.; Ellis, L.M. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2005**, *23*, 1011-1027, doi:10.1200/jco.2005.06.081.
90. Carmeliet, P.; Jain, R.K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249, doi:10.1038/35025220.
91. Thomas, A.; Pattanayak, P.; Szabo, E.; Pinsky, P. Characteristics and Outcomes of Small Cell Lung Cancer Detected by CT Screening. *Chest* **2018**, *154*, 1284-1290, doi:<https://doi.org/10.1016/j.chest.2018.07.029>.
92. Hodgkinson, C.L.; Morrow, C.J.; Li, Y.; Metcalf, R.L.; Rothwell, D.G.; Trapani, F.; Polanski, R.; Burt, D.J.; Simpson, K.L.; Morris, K., et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. *Nature medicine* **2014**, *20*, 897-903, doi:10.1038/nm.3600

<http://www.nature.com/nm/journal/v20/n8/abs/nm.3600.html#supplementary-information>.

93. Hamilton, G.; Moser, D.; Hochmair, M. Metastasis: Circulating Tumor Cells in Small Cell Lung Cancer. *Trends in Cancer* **2016**, *2*, 159-160, doi:10.1016/j.trecan.2016.02.006.
94. Lucchi, M.; Mussi, A.; Fontanini, G.; Faviana, P.; Ribechini, A.; Angeletti, C.A. Small cell lung carcinoma (SCLC): the angiogenic phenomenon. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery* **2002**, *21*, 1105-1110.
95. Dowell, J.E.; Amirkhan, R.H.; Lai, W.S.; Frawley, W.H.; Minna, J.D. Survival in small cell lung cancer is independent of tumor expression of VEGF and COX-2. *Anticancer research* **2004**, *24*, 2367-2373.
96. Salven, P.; Ruotsalainen, T.; Mattson, K.; Joensuu, H. High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. *International Journal of Cancer* **1998**, *79*, 144-146, doi:10.1002/(sici)1097-0215(19980417)79:2<144::aid-ijc8>3.0.co;2-t.
97. Tas, F.; Duranyildiz, D.; Oguz, H.; Camlica, H.; Yasasever, V.; Topuz, E. Serum vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) levels in small cell lung cancer. *Cancer Invest* **2006**, *24*, 492-496, doi:10.1080/07357900600814771.
98. Hasegawa, Y.; Takanashi, S.; Okudera, K.; Kumagai, M.; Hayashi, A.; Morimoto, T.; Okumura, K. Vascular endothelial growth factor level as a prognostic determinant of small cell lung cancer in Japanese patients. *Internal medicine* **2005**, *44*, 26-34, doi:10.2169/internalmedicine.44.26.
99. Zhan, P.; Wang, J.; Lv, X.J.; Wang, Q.; Qiu, L.X.; Lin, X.Q.; Yu, L.K.; Song, Y. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. *J Thorac Oncol* **2009**, *4*, 1094-1103, doi:10.1097/JTO.0b013e3181a97e31.
100. Ustuner, Z.; Saip, P.; Yasasever, V.; Vural, B.; Yazar, A.; Bal, C.; Ozturk, B.; Ozbek, U.; Topuz, E. Prognostic and predictive value of vascular endothelial growth factor and its soluble receptors, VEGFR-1 and VEGFR-2 levels in the sera of small cell lung cancer patients. *Medical oncology (Northwood, London, England)* **2008**, *25*, 394-399, doi:10.1007/s12032-008-9052-4.
101. Zhang, C.L.; Ge, S.L.; Yang, N.; Zhang, J.R.; Tian, D.D. Elevated Serum Level of Angiopoietin-2 as a Potential Marker for Poor Prognosis in Small Cell Lung Cancer. *Tohoku J Exp Med* **2015**, *236*, 305-309, doi:10.1620/tjem.236.305.
102. Cañadas, I.; Taus, Á.; Villanueva, X.; Arpí, O.; Pijuan, L.; Rodríguez, Y.; Menéndez, S.; Mojal, S.; Rojo, F.; Albanell, J., et al. Angiopoietin-2 is a negative prognostic marker in small cell lung cancer. *Lung cancer* **2015**, *90*, 302-306, doi:10.1016/j.lungcan.2015.09.023.

103. Holash, J.; Davis, S.; Papadopoulos, N.; Croll, S.D.; Ho, L.; Russell, M.; Boland, P.; Leidich, R.; Hylton, D.; Burova, E., et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 11393-11398, doi:10.1073/pnas.172398299.
104. Ryan, A.J.; Wedge, S.R. ZD6474--a novel inhibitor of VEGFR and EGFR tyrosine kinase activity. *British journal of cancer* **2005**, *92 Suppl 1*, S6-13, doi:10.1038/sj.bjc.6602603.
105. O'Farrell, A.M.; Abrams, T.J.; Yuen, H.A.; Ngai, T.J.; Louie, S.G.; Yee, K.W.; Wong, L.M.; Hong, W.; Lee, L.B.; Town, A., et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood* **2003**, *101*, 3597-3605, doi:10.1182/blood-2002-07-2307.
106. Wedge, S.R.; Kendrew, J.; Hennequin, L.F.; Valentine, P.J.; Barry, S.T.; Brave, S.R.; Smith, N.R.; James, N.H.; Dukes, M.; Curwen, J.O., et al. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. *Cancer research* **2005**, *65*, 4389-4400, doi:10.1158/0008-5472.Can-04-4409.
107. Wilhelm, S.M.; Carter, C.; Tang, L.; Wilkie, D.; McNabola, A.; Rong, H.; Chen, C.; Zhang, X.; Vincent, P.; McHugh, M., et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer research* **2004**, *64*, 7099-7109, doi:10.1158/0008-5472.Can-04-1443.
108. Hilberg, F.; Roth, G.J.; Krssak, M.; Kautschitsch, S.; Sommergruber, W.; Tontsch-Grunt, U.; Garin-Chesa, P.; Bader, G.; Zoephel, A.; Quant, J., et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer research* **2008**, *68*, 4774-4782, doi:10.1158/0008-5472.Can-07-6307.
109. D'Amato, R.J.; Loughnan, M.S.; Flynn, E.; Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 4082-4085, doi:10.1073/pnas.91.9.4082.
110. Sonpavde, G.; Hutson, T.E. Pazopanib: a novel multitargeted tyrosine kinase inhibitor. *Curr Oncol Rep* **2007**, *9*, 115-119, doi:10.1007/s11912-007-0007-2.
111. Ready, N.E.; Dudek, A.Z.; Pang, H.H.; Hodgson, L.D.; Graziano, S.L.; Green, M.R.; Vokes, E.E. Cisplatin, Irinotecan, and Bevacizumab for Untreated Extensive-Stage Small-Cell Lung Cancer: CALGB 30306, a Phase II Study. *Journal of Clinical Oncology* **2011**, *29*, 4436-4441, doi:10.1200/jco.2011.35.6923.
112. Spigel, D.R.; Townley, P.M.; Waterhouse, D.M.; Fang, L.; Adiguzel, I.; Huang, J.E.; Karlin, D.A.; Faoro, L.; Scappaticci, F.A.; Socinski, M.A. Randomized phase II study of bevacizumab in combination with chemotherapy in previously untreated extensive-stage small-cell lung cancer: results from the SALUTE trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2011**, *29*, 2215-2222, doi:10.1200/JCO.2010.29.3423.

113. Horn, L.; Dahlberg, S.E.; Sandler, A.B.; Dowlati, A.; Moore, D.F.; Murren, J.R.; Schiller, J.H. Phase II study of cisplatin plus etoposide and bevacizumab for previously untreated, extensive-stage small-cell lung cancer: Eastern Cooperative Oncology Group Study E3501. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2009**, *27*, 6006-6011, doi:10.1200/jco.2009.23.7545.
114. Spigel, D.R.; Townley, P.M.; Waterhouse, D.M.; Fang, L.; Adiguzel, I.; Huang, J.E.; Karlin, D.A.; Faoro, L.; Scappaticci, F.A.; Socinski, M.A. Randomized Phase II Study of Bevacizumab in Combination With Chemotherapy in Previously Untreated Extensive-Stage Small-Cell Lung Cancer: Results From the SALUTE Trial. *Journal of Clinical Oncology* **2011**, *29*, 2215-2222, doi:10.1200/jco.2010.29.3423.
115. Pujol, J.L.; Lavole, A.; Quoix, E.; Molinier, O.; Souquet, P.J.; Barlesi, F.; Le Caer, H.; Moro-Sibilot, D.; Fournel, P.; Oster, J.P., et al. Randomized phase II-III study of bevacizumab in combination with chemotherapy in previously untreated extensive small-cell lung cancer: results from the IFCT-0802 trial†. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2015**, *26*, 908-914, doi:10.1093/annonc/mdv065.
116. Petrioli, R.; Roviello, G.; Laera, L.; Luzzi, L.; Paladini, P.; Ghiribelli, C.; Voltolini, L.; Martellucci, I.; Bianco, V.; Francini, E. Cisplatin, Etoposide, and Bevacizumab Regimen Followed by Oral Etoposide and Bevacizumab Maintenance Treatment in Patients With Extensive-Stage Small Cell Lung Cancer: A Single-Institution Experience. *Clin Lung Cancer* **2015**, *16*, e229-234, doi:10.1016/j.clc.2015.05.005.
117. Jalal, S.; Bedano, P.; Einhorn, L.; Bhatia, S.; Ansari, R.; Bechar, N.; Koneru, K.; Govindan, R.; Wu, J.; Yu, M., et al. Paclitaxel plus bevacizumab in patients with chemosensitive relapsed small cell lung cancer: a safety, feasibility, and efficacy study from the Hoosier Oncology Group. *J Thorac Oncol* **2010**, *5*, 2008-2011, doi:10.1097/JTO.0b013e3181f77b6e.
118. Mountzios, G.; Emmanouilidis, C.; Vardakis, N.; Kontopodis, E.; Hatzidaki, D.; Popis, E.; Karachaliou, N.; Kotsakis, A.; Agelidou, M.; Georgoulas, V. Paclitaxel plus bevacizumab in patients with chemoresistant relapsed small cell lung cancer as salvage treatment: a phase II multicenter study of the Hellenic Oncology Research Group. *Lung cancer* **2012**, *77*, 146-150, doi:10.1016/j.lungcan.2012.02.002.
119. Spigel, D.R.; Waterhouse, D.M.; Lane, S.; Legenne, P.; Bhatt, K. Efficacy and safety of oral topotecan and bevacizumab combination as second-line treatment for relapsed small-cell lung cancer: an open-label multicenter single-arm phase II study. *Clin Lung Cancer* **2013**, *14*, 356-363, doi:10.1016/j.clc.2012.12.003.
120. Schneider, B.J.; Gadgil, S.M.; Ramnath, N.; Wozniak, A.J.; Dy, G.K.; Daignault, S.; Kalemkerian, G.P. Phase II trial of sunitinib maintenance therapy after platinum-based chemotherapy in patients with extensive-stage small cell lung cancer. *J Thorac Oncol* **2011**, *6*, 1117-1120, doi:10.1097/JTO.0b013e31821529c3.

121. Spigel, D.R.; Greco, F.A.; Rubin, M.S.; Shipley, D.; Thompson, D.S.; Lubiner, E.T.; Eakle, J.F.; Quinn, R.; Burris, H.A.; Hainsworth, J.D. Phase II study of maintenance sunitinib following irinotecan and carboplatin as first-line treatment for patients with extensive-stage small-cell lung cancer. *Lung cancer* **2012**, *77*, 359-364, doi:10.1016/j.lungcan.2012.03.009.
122. Ready, N.E.; Pang, H.H.; Gu, L.; Otterson, G.A.; Thomas, S.P.; Miller, A.A.; Baggstrom, M.; Masters, G.A.; Graziano, S.L.; Crawford, J., et al. Chemotherapy With or Without Maintenance Sunitinib for Untreated Extensive-Stage Small-Cell Lung Cancer: A Randomized, Double-Blind, Placebo-Controlled Phase II Study-CALGB 30504 (Alliance). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2015**, *33*, 1660-1665, doi:10.1200/jco.2014.57.3105.
123. Han, J.Y.; Kim, H.Y.; Lim, K.Y.; Han, J.H.; Lee, Y.J.; Kwak, M.H.; Kim, H.J.; Yun, T.; Kim, H.T.; Lee, J.S. A phase II study of sunitinib in patients with relapsed or refractory small cell lung cancer. *Lung cancer* **2013**, *79*, 137-142, doi:10.1016/j.lungcan.2012.09.019.
124. Lu, S.; Li, L.; Luo, Y.; Zhang, L.; Wu, G.; Chen, Z.; Huang, C.; Guo, S.; Zhang, Y.; Song, X., et al. A Multicenter, Open-Label, Randomized Phase II Controlled Study of rh-Endostatin (Endostar) in Combination with Chemotherapy in Previously Untreated Extensive-Stage Small-Cell Lung Cancer. *Journal of Thoracic Oncology* **2015**, *10*, 206-211, doi:10.1097/JTO.0000000000000343.
125. Pujol, J.L.; Breton, J.L.; Gervais, R.; Tanguy, M.L.; Quoix, E.; David, P.; Janicot, H.; Westeel, V.; Gamaroff, S.; Genève, J., et al. Phase III double-blind, placebo-controlled study of thalidomide in extensive-disease small-cell lung cancer after response to chemotherapy: an intergroup study FNCLCC cleo04 IFCT 00-01. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2007**, *25*, 3945-3951, doi:10.1200/jco.2007.11.8109.
126. Lee, S.M.; Woll, P.J.; Rudd, R.; Ferry, D.; O'Brien, M.; Middleton, G.; Spiro, S.; James, L.; Ali, K.; Jitlal, M., et al. Anti-angiogenic therapy using thalidomide combined with chemotherapy in small cell lung cancer: a randomized, double-blind, placebo-controlled trial. *Journal of the National Cancer Institute* **2009**, *101*, 1049-1057, doi:10.1093/jnci/djp200.
127. Allen, J.W.; Moon, J.; Redman, M.; Gadgeel, S.M.; Kelly, K.; Mack, P.C.; Saba, H.M.; Mohamed, M.K.; Jahanzeb, M.; Gandara, D.R. Southwest Oncology Group S0802: a randomized, phase II trial of weekly topotecan with and without ziv-aflibercept in patients with platinum-treated small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2014**, *32*, 2463-2470, doi:10.1200/jco.2013.51.4109.
128. Arnold, A.M.; Seymour, L.; Smylie, M.; Ding, K.; Ung, Y.; Findlay, B.; Lee, C.W.; Djurfeldt, M.; Whitehead, M.; Ellis, P., et al. Phase II study of vandetanib or placebo in small-cell lung cancer patients after complete or partial response to induction chemotherapy with or without radiation therapy: National Cancer Institute of Canada Clinical Trials Group Study BR.20. *Journal of clinical oncology : official*

journal of the American Society of Clinical Oncology **2007**, 25, 4278-4284, doi:10.1200/jco.2007.12.3083.

129. Ramalingam, S.S.; Belani, C.P.; Mack, P.C.; Vokes, E.E.; Longmate, J.; Govindan, R.; Koczywas, M.; Ivy, S.P.; Gandara, D.R. Phase II study of Cediranib (AZD 2171), an inhibitor of the vascular endothelial growth factor receptor, for second-line therapy of small cell lung cancer (National Cancer Institute #7097). *J Thorac Oncol* **2010**, 5, 1279-1284, doi:10.1097/JTO.0b013e3181e2fcb0.

130. Gitlitz, B.J.; Moon, J.; Glisson, B.S.; Reimers, H.J.; Bury, M.J.; Floyd, J.D.; Schulz, T.K.; Sundaram, P.K.; Ho, C.; Gandara, D.R. Sorafenib in platinum-treated patients with extensive stage small cell lung cancer: a Southwest Oncology Group (SWOG 0435) phase II trial. *J Thorac Oncol* **2010**, 5, 1835-1840, doi:10.1097/JTO.0b013e3181f0bd78.

131. Koinis, F.; Agelaki, S.; Karavassilis, V.; Kentepozidis, N.; Samantas, E.; Peroukidis, S.; Katsaounis, P.; Hartabilas, E.; Varthalitis, I.; Messaritakis, I., et al. Second-line pazopanib in patients with relapsed and refractory small-cell lung cancer: a multicentre phase II study of the Hellenic Oncology Research Group. *British journal of cancer* **2017**, 117, 8-14, doi:10.1038/bjc.2017.137.

132. Han, J.Y.; Kim, H.Y.; Lim, K.Y.; Hwangbo, B.; Lee, J.S. A phase II study of nintedanib in patients with relapsed small cell lung cancer. *Lung cancer* **2016**, 96, 108-112, doi:10.1016/j.lungcan.2016.04.002.

133. Stratigos, M.; Matikas, A.; Voutsina, A.; Mavroudis, D.; Georgoulas, V. Targeting angiogenesis in small cell lung cancer. *Translational lung cancer research* **2016**, 5, 389-400, doi:10.21037/tlcr.2016.08.04.

134. Aberle, D.R.; Adams, A.M.; Berg, C.D.; Black, W.C.; Clapp, J.D.; Fagerstrom, R.M.; Gareen, I.F.; Gatsonis, C.; Marcus, P.M.; Sicks, J.D. Reduced lung-cancer mortality with low-dose computed tomographic screening. *The New England journal of medicine* **2011**, 365, 395-409, doi:10.1056/NEJMoa1102873.

135. Pastorino, U.; Silva, M.; Sestini, S.; Sabia, F.; Boeri, M.; Cantarutti, A.; Sverzellati, N.; Sozzi, G.; Corrao, G.; Marchianò, A. Prolonged lung cancer screening reduced 10-year mortality in the MILD trial: new confirmation of lung cancer screening efficacy. *Annals of Oncology* **2019**, 30, 1162-1169, doi:10.1093/annonc/mdz117.

136. De Koning H, V.D.A.C., Ten Haaf K, et al. Effects of volume CT lung cancer screening: Mortality results of the NELSON randomized-controlled population based trial. *2018 World Conference on Lung Cancer. Abstract PL02.05*.

137. de Koning, H.J.; van der Aalst, C.M.; de Jong, P.A.; Scholten, E.T.; Nackaerts, K.; Heuvelmans, M.A.; Lammers, J.-W.J.; Weenink, C.; Yousaf-Khan, U.; Horeweg, N., et al. Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. *New England Journal of Medicine* **2020**, 10.1056/NEJMoa1911793, doi:10.1056/NEJMoa1911793.

138. Travis, W.D. Advances in neuroendocrine lung tumors. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2010**, *21 Suppl 7*, vii65-71, doi:10.1093/annonc/mdq380.
139. Bertino, E.M.; Confer, P.D.; Colonna, J.E.; Ross, P.; Otterson, G.A. Pulmonary neuroendocrine/carcinoid tumors: a review article. *Cancer* **2009**, *115*, 4434-4441, doi:10.1002/cncr.24498.
140. Hendifar, A.E.; Marchevsky, A.M.; Tuli, R. Neuroendocrine Tumors of the Lung: Current Challenges and Advances in the Diagnosis and Management of Well-Differentiated Disease. *J Thorac Oncol* **2017**, *12*, 425-436, doi:10.1016/j.jtho.2016.11.2222.
141. Travis, W.D. Pathology and diagnosis of neuroendocrine tumors: lung neuroendocrine. *Thoracic surgery clinics* **2014**, *24*, 257-266, doi:10.1016/j.thorsurg.2014.04.001.
142. Caplin, M.E.; Baudin, E.; Ferolla, P.; Filosso, P.; Garcia-Yuste, M.; Lim, E.; Oberg, K.; Pelosi, G.; Perren, A.; Rossi, R.E., et al. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2015**, *26*, 1604-1620, doi:10.1093/annonc/mdv041.
143. Khuder, S.A. Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. *Lung cancer* **2001**, *31*, 139-148, doi:[https://doi.org/10.1016/S0169-5002\(00\)00181-1](https://doi.org/10.1016/S0169-5002(00)00181-1).
144. Froudarakis, M.; Fournel, P.; Burgard, G.; Bouros, D.; Boucheron, S.; Siafakas, N.M.; Emonot, A. Bronchial Carcinoids. *Oncology* **1996**, *53*, 153-158, doi:10.1159/000227552.
145. Beasley, M.B.; Thunnissen, F.B.J.M.; Brambilla, E.; Hasleton, P.; Steele, R.; Hammar, S.P.; Colby, T.V.; Sheppard, M.; Shimosato, Y.; Koss, M.N., et al. Pulmonary atypical carcinoid: Predictors of survival in 106 cases. *Human pathology* **2000**, *31*, 1255-1265, doi:<https://doi.org/10.1053/hupa.2000.19294>.
146. Fink, G.; Krelbaum, T.; Yellin, A.; Bendayan, D.; Saute, M.; Glazer, M.; Kramer, M.R. Pulmonary Carcinoid: Presentation, Diagnosis, and Outcome in 142 Cases in Israel and Review of 640 Cases From the Literature. *Chest* **2001**, *119*, 1647-1651, doi:<https://doi.org/10.1378/chest.119.6.1647>.
147. Faivre-Finn, C.; Snee, M.; Ashcroft, L.; Appel, W.; Barlesi, F.; Bhatnagar, A.; Bezjak, A.; Cardenal, F.; Fournel, P.; Harden, S., et al. Concurrent once-daily versus twice-daily chemoradiotherapy in patients with limited-stage small-cell lung cancer (CONVERT): an open-label, phase 3, randomised, superiority trial. *The Lancet Oncology* **2017**, *18*, 1116-1125, doi:10.1016/S1470-2045(17)30318-2.
148. George, J.; Lim, J.S.; Jang, S.J.; Cun, Y.; Ozretic, L.; Kong, G.; Leenders, F.; Lu, X.; Fernandez-Cuesta, L.; Bosco, G., et al. Comprehensive genomic profiles of small cell lung cancer. *Nature* **2015**, *524*, 47-53, doi:10.1038/nature14664.

149. Sutherland, Kate D.; Proost, N.; Brouns, I.; Adriaensen, D.; Song, J.-Y.; Berns, A. Cell of Origin of Small Cell Lung Cancer: Inactivation of Trp53 and Rb1 in Distinct Cell Types of Adult Mouse Lung. *Cancer cell* **2011**, *19*, 754-764, doi:<https://doi.org/10.1016/j.ccr.2011.04.019>.
150. Recondo, G.; Facchinetti, F.; Olaussen, K.A.; Besse, B.; Friboulet, L. Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? *Nat Rev Clin Oncol* **2018**, 10.1038/s41571-018-0081-4, doi:10.1038/s41571-018-0081-4.
151. Ross, J.S.; Wang, K.; Elkadi, O.R.; Tarasen, A.; Foulke, L.; Sheehan, C.E.; Otto, G.A.; Palmer, G.; Yelensky, R.; Lipson, D., et al. Next-generation sequencing reveals frequent consistent genomic alterations in small cell undifferentiated lung cancer. *Journal of Clinical Pathology* **2014**, *67*, 772, doi:10.1136/jclinpath-2014-202447.
152. Vogelstein, B.; Papadopoulos, N.; Velculescu, V.E.; Zhou, S.; Diaz, L.A.; Kinzler, K.W. Cancer Genome Landscapes. *Science (New York, N.Y.)* **2013**, *339*, 1546-1558, doi:10.1126/science.1235122.
153. Peifer, M.; Fernandez-Cuesta, L.; Sos, M.L.; George, J.; Seidel, D.; Kasper, L.H.; Plenker, D.; Leenders, F.; Sun, R.; Zander, T., et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nature genetics* **2012**, *44*, 1104-1110, doi:10.1038/ng.2396.
154. Rudin, C.M.; Durinck, S.; Stawiski, E.W.; Poirier, J.T.; Modrusan, Z.; Shames, D.S.; Bergbower, E.A.; Guan, Y.; Shin, J.; Guillory, J., et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. *Nature genetics* **2012**, *44*, 1111-1116, doi:10.1038/ng.2405.
155. Yokomizo, A.; Tindall, D.J.; Drabkin, H.; Gemmill, R.; Franklin, W.; Yang, P.; Sugio, K.; Smith, D.I.; Liu, W. PTEN/MMAC1 mutations identified in small cell, but not in non-small cell lung cancers. *Oncogene* **1998**, *17*, 475-479, doi:10.1038/sj.onc.1201956.
156. Devarakonda, S.; Morgensztern, D.; Govindan, R. Genomic alterations in lung adenocarcinoma. *The Lancet Oncology* **2015**, *16*, e342-e351, doi:[http://dx.doi.org/10.1016/S1470-2045\(15\)00077-7](http://dx.doi.org/10.1016/S1470-2045(15)00077-7).
157. Toyooka, S.; Tsuda, T.; Gazdar, A.F. The TP53 gene, tobacco exposure, and lung cancer. *Human mutation* **2003**, *21*, 229-239, doi:doi:10.1002/humu.10177.
158. Meuwissen, R.; Linn, S.C.; Linnoila, R.I.; Zevenhoven, J.; Mooi, W.J.; Berns, A. Induction of small cell lung cancer by somatic inactivation of both *Trp53* and *Rb1* in a conditional mouse model. *Cancer cell* **2003**, *4*, 181-189, doi:10.1016/S1535-6108(03)00220-4.
159. OKim, D.-W.; Kim, K.-C.; Kim, K.-B.; Dunn, C.T.; Park, K.-S. Transcriptional deregulation underlying the pathogenesis of small cell lung cancer. *Translational Lung Cancer Research* **2017**, *7*, 4-20.

160. Hiroshima, K.; Iyoda, A.; Shida, T.; Shibuya, K.; Iizasa, T.; Kishi, H.; Tanizawa, T.; Fujisawa, T.; Nakatani, Y. Distinction of pulmonary large cell neuroendocrine carcinoma from small cell lung carcinoma: a morphological, immunohistochemical, and molecular analysis. *Mod Pathol* **2006**, *19*, 1358-1368, doi:10.1038/modpathol.3800659.
161. Gazdar, A.F.; Carney, D.N.; Nau, M.M.; Minna, J.D. Characterization of Variant Subclasses of Cell Lines Derived from Small Cell Lung Cancer Having Distinctive Biochemical, Morphological, and Growth Properties. *Cancer research* **1985**, *45*, 2924.
162. Travis, W.D. Lung tumours with neuroendocrine differentiation. *European Journal of Cancer* **2009**, *45*, 251-266, doi:[https://doi.org/10.1016/S0959-8049\(09\)70040-1](https://doi.org/10.1016/S0959-8049(09)70040-1).
163. Borromeo, Mark D.; Savage, Trisha K.; Kollipara, Rahul K.; He, M.; Augustyn, A.; Osborne, Jihan K.; Girard, L.; Minna, John D.; Gazdar, Adi F.; Cobb, Melanie H., et al. ASCL1 and NEUROD1 Reveal Heterogeneity in Pulmonary Neuroendocrine Tumors and Regulate Distinct Genetic Programs. *Cell Reports* **2016**, *16*, 1259-1272, doi:<https://doi.org/10.1016/j.celrep.2016.06.081>.
164. Rudin, C.M.; Poirier, J.T.; Byers, L.A.; Dive, C.; Dowlati, A.; George, J.; Heymach, J.V.; Johnson, J.E.; Lehman, J.M.; MacPherson, D., et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nature reviews. Cancer* **2019**, 10.1038/s41568-019-0133-9, doi:10.1038/s41568-019-0133-9.
165. Huang, Y.H.; Klingbeil, O.; He, X.Y.; Wu, X.S.; Arun, G.; Lu, B.; Somerville, T.D.D.; Milazzo, J.P.; Wilkinson, J.E.; Demerdash, O.E., et al. POU2F3 is a master regulator of a tuft cell-like variant of small cell lung cancer. *Genes Dev* **2018**, *32*, 915-928, doi:10.1101/gad.314815.118.
166. McColl, K.; Wildey, G.; Sakre, N.; Lipka, M.B.; Behtaj, M.; Kresak, A.; Chen, Y.; Yang, M.; Velcheti, V.; Fu, P., et al. Reciprocal expression of INSM1 and YAP1 defines subgroups in small cell lung cancer. *Oncotarget* **2017**, *8*, 73745-73756, doi:10.18632/oncotarget.20572.
167. Hirsch, M.R.; Tiveron, M.C.; Guillemot, F.; Brunet, J.F.; Goriadis, C. Control of noradrenergic differentiation and Phox2a expression by MASH1 in the central and peripheral nervous system. *Development* **1998**, *125*, 599.
168. Casarosa, S.; Fode, C.; Guillemot, F. Mash1 regulates neurogenesis in the ventral telencephalon. *Development* **1999**, *126*, 525.
169. Hevner, R.F.; Hodge, R.D.; Daza, R.A.; Englund, C. Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. *Neuroscience research* **2006**, *55*, 223-233, doi:10.1016/j.neures.2006.03.004.

170. Aprea, J.; Nonaka-Kinoshita, M.; Calegari, F. Generation and characterization of NeuroD1-CreERT2 mouse lines for the study of embryonic and adult neurogenesis. *genesis* **2014**, *52*, 870-878, doi:doi:10.1002/dvg.22797.
171. Pataskar, A.; Jung, J.; Smialowski, P.; Noack, F.; Calegari, F.; Straub, T.; Tiwari, V.K. NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program. *The EMBO journal* **2016**, *35*, 24-45, doi:doi:10.15252/embj.201591206.
172. Vierbuchen, T.; Ostermeier, A.; Pang, Z.P.; Kokubu, Y.; Sudhof, T.C.; Wernig, M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **2010**, *463*, 1035-1041, doi:10.1038/nature08797.
173. Borges, M.; Linnoila, R.I.; van de Velde, H.J.K.; Chen, H.; Nelkin, B.D.; Mabry, M.; Baylin, S.B.; Ball, D.W. An achaete-scute homologue essential for neuroendocrine differentiation in the lung. *Nature* **1997**, *386*, 852, doi:10.1038/386852a0.
174. Neptune, E.R.; Podowski, M.; Calvi, C.; Cho, J.-H.; Garcia, J.G.N.; Tuder, R.; Linnoila, R.I.; Tsai, M.-J.; Dietz, H.C. Targeted disruption of NeuroD, a proneural basic helix-loop-helix factor, impairs distal lung formation and neuroendocrine morphology in the neonatal lung. *The Journal of biological chemistry* **2008**, *283*, 21160-21169, doi:10.1074/jbc.M708692200.
175. Cindolo, L.; Franco, R.; Cantile, M.; Schiavo, G.; Liguori, G.; Chiodini, P.; Salzano, L.; Autorino, R.; Di Blasi, A.; Falsaperla, M., et al. NeuroD1 Expression in Human Prostate Cancer: Can It Contribute to Neuroendocrine Differentiation Comprehension? *European Urology* **2007**, *52*, 1365-1373, doi:10.1016/j.eururo.2006.11.030.
176. Shida, T.; Furuya, M.; Kishimoto, T.; Nikaido, T.; Tanizawa, T.; Koda, K.; Oda, K.; Takano, S.; Kimura, F.; Shimizu, H., et al. The expression of NeuroD and mASH1 in the gastroenteropancreatic neuroendocrine tumors. *Modern Pathology* **2008**, *21*, 1363, doi:10.1038/modpathol.2008.121.
177. Ferretti, E.; Di Stefano, D.; Zazzeroni, F.; Gallo, R.; Fratticci, A.; Carfagnini, R.; Angiulli, S.; Santoro, A.; Minniti, G.; Tamburrano, G., et al. Human pituitary tumours express the bHLH transcription factors NeuroD1 and ASH1. *J Endocrinol Invest* **2003**, *26*, 957-965, doi:10.1007/bf03348192.
178. Shida, T.; Furuya, M.; Nikaido, T.; Kishimoto, T.; Koda, K.; Oda, K.; Nakatani, Y.; Miyazaki, M.; Ishikura, H. Aberrant expression of human achaete-scute homologue gene 1 in the gastrointestinal neuroendocrine carcinomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2005**, *11*, 450-458.
179. Osborne, J.K.; Larsen, J.E.; Gonzales, J.X.; Shames, D.S.; Sato, M.; Wistuba, I.I.; Girard, L.; Minna, J.D.; Cobb, M.H. NeuroD1 regulation of migration accompanies the differential sensitivity of neuroendocrine carcinomas to TrkB inhibition. *Oncogenesis* **2013**, *2*, e63, doi:10.1038/oncsis.2013.24

<https://www.nature.com/articles/oncsis201324#supplementary-information>.

180. Osborne, J.K.; Larsen, J.E.; Shields, M.D.; Gonzales, J.X.; Shames, D.S.; Sato, M.; Kulkarni, A.; Wistuba, I.I.; Girard, L.; Minna, J.D., et al. NeuroD1 regulates survival and migration of neuroendocrine lung carcinomas via signaling molecules TrkB and NCAM. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 6524-6529, doi:10.1073/pnas.1303932110.
181. Meder, L.; Konig, K.; Ozretic, L.; Schultheis, A.M.; Ueckerth, F.; Ade, C.P.; Albus, K.; Boehm, D.; Rommerscheidt-Fuss, U.; Florin, A., et al. NOTCH, ASCL1, p53 and RB alterations define an alternative pathway driving neuroendocrine and small cell lung carcinomas. *International journal of cancer. Journal international du cancer* **2015**, 10.1002/ijc.29835, doi:10.1002/ijc.29835.
182. Chapman, G.; Sparrow, D.B.; Kremmer, E.; Dunwoodie, S.L. Notch inhibition by the ligand Delta-Like 3 defines the mechanism of abnormal vertebral segmentation in spondylocostal dysostosis. *Human Molecular Genetics* **2011**, *20*, 905-916, doi:10.1093/hmg/ddq529.
183. Ladi, E.; Nichols, J.T.; Ge, W.; Miyamoto, A.; Yao, C.; Yang, L.-T.; Boulter, J.; Sun, Y.E.; Kintner, C.; Weinmaster, G. The divergent DSL ligand DLL3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *The Journal of cell biology* **2005**, *170*, 983-992, doi:10.1083/jcb.200503113.
184. Sriuranpong, V.; Borges, M.W.; Strock, C.L.; Nakakura, E.K.; Watkins, D.N.; Blaumueller, C.M.; Nelkin, B.D.; Ball, D.W. Notch signaling induces rapid degradation of achaete-scute homolog 1. *Molecular and cellular biology* **2002**, *22*, 3129-3139, doi:10.1128/MCB.22.9.3129-3139.2002.
185. Saunders, L.R.; Bankovich, A.J.; Anderson, W.C.; Aujay, M.A.; Bheddah, S.; Black, K.; Desai, R.; Escarpe, P.A.; Hampl, J.; Laysang, A., et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Science translational medicine* **2015**, *7*, 302ra136.
186. Carbone, D.P.; Morgensztern, D.; Moulec, S.L.; Santana-Davila, R.; Ready, N.; Hann, C.L.; Glisson, B.S.; Dowlati, A.; Rudin, C.M.; Lally, S., et al. Efficacy and safety of rovalpituzumab tesirine in patients With DLL3-expressing, $\geq$ 3rd line small cell lung cancer: Results from the phase 2 TRINITY study. *Journal of Clinical Oncology* **2018**, *36*, 8507-8507, doi:10.1200/JCO.2018.36.15_suppl.8507.
187. AbbVie. Phase 3 Trial of Rova-T as Second-line Therapy for Advanced Small-Cell Lung Cancer (TAHOE Study) Halted. *AbbVie*. <https://bit.ly/2KZcyVI>. Accessed January 4, 2019. **Published December 5, 2018**.
188. Nowell, C.S.; Radtke, F. Notch as a tumour suppressor. *Nature Reviews Cancer* **2017**, *17*, 145-159, doi:10.1038/nrc.2016.145.
189. Mollaoglu, G.; Guthrie, M.R.; Böhm, S.; Brägelmann, J.; Can, I.; Ballieu, P.M.; Marx, A.; George, J.; Heinen, C.; Chalishazar, M.D., et al. MYC Drives Progression of Small Cell Lung Cancer to a Variant Neuroendocrine Subtype with

Vulnerability to Aurora Kinase Inhibition. *Cancer cell* **2017**, *31*, 270-285, doi:10.1016/j.ccell.2016.12.005.

190. Sulzmaier, F.J.; Jean, C.; Schlaepfer, D.D. FAK in cancer: mechanistic findings and clinical applications. *Nature reviews. Cancer* **2014**, *14*, 598-610, doi:10.1038/nrc3792.

191. Carelli, S.; Zadra, G.; Vaira, V.; Falleni, M.; Bottiglieri, L.; Nosotti, M.; Di Giulio, A.M.; Gorio, A.; Bosari, S. Up-regulation of focal adhesion kinase in non-small cell lung cancer. *Lung cancer* **2006**, *53*, 263-271, doi:10.1016/j.lungcan.2006.06.001.

192. Dy, G.K.; Ylagan, L.; Pokharel, S.; Miller, A.; Brese, E.; Bshara, W.; Morrison, C.; Cance, W.G.; Golubovskaya, V.M. The Prognostic Significance of Focal Adhesion Kinase Expression in Stage I Non-Small-Cell Lung Cancer. *Journal of Thoracic Oncology* **2014**, *9*, 1278-1284, doi:<https://doi.org/10.1097/JTO.0000000000000248>.

193. Wang, C.; Yang, R.; Yue, D.; Zhang, Z. Expression of FAK and PTEN in Bronchioloalveolar Carcinoma and Lung Adenocarcinoma. *Lung* **2009**, *187*, 104-109, doi:10.1007/s00408-008-9130-6.

194. Imaizumi, M.; Nishimura, M.; Takeuchi, S.; Murase, M.; Hamaguchi, M. Role of tyrosine specific phosphorylation of cellular proteins, especially EGF receptor and p125FAK in human lung cancer cells. *Lung cancer* **1997**, *17*, 69-84, doi:10.1016/S0169-5002(97)00650-8.

195. Ocak, S.; Chen, H.; Callison, C.; Gonzalez, A.L.; Massion, P.P. Expression of focal adhesion kinase in small-cell lung carcinoma. *Cancer* **2012**, *118*, 1293-1301, doi:10.1002/cncr.26382.

196. Hsu, N.Y.; Chen, C.Y.; Hsu, C.P.; Lin, T.Y.; Chou, M.C.; Chiou, S.H.; Chow, K.C. Prognostic significance of expression of nm23-H1 and focal adhesion kinase in non-small cell lung cancer. *Oncology reports* **2007**, *18*, 81-85.

197. Ji, H.F.; Pang, D.; Fu, S.B.; Jin, Y.; Yao, L.; Qi, J.P.; Bai, J. Overexpression of focal adhesion kinase correlates with increased lymph node metastasis and poor prognosis in non-small-cell lung cancer. *Journal of cancer research and clinical oncology* **2013**, *139*, 429-435, doi:10.1007/s00432-012-1342-8.

198. Owens, L.V.; Xu, L.; Craven, R.J.; Dent, G.A.; Weiner, T.M.; Kornberg, L.; Liu, E.T.; Cance, W.G. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer research* **1995**, *55*, 2752-2755.

199. Ocak, S.; Yamashita, H.; Udyavar, A.R.; Miller, A.N.; Gonzalez, A.L.; Zou, Y.; Jiang, A.; Yi, Y.; Shyr, Y.; Estrada, L., et al. DNA copy number aberrations in small-cell lung cancer reveal activation of the focal adhesion pathway. *Oncogene* **2010**, *29*, 6331-6342, doi:10.1038/onc.2010.362.

200. Blume-Jensen, P.; Hunter, T. Oncogenic kinase signalling. *Nature* **2001**, *411*, 355, doi:10.1038/35077225.

201. Schaller, M.D.; Borgman, C.A.; Parsons, J.T. Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125FAK. *Molecular and cellular biology* **1993**, *13*, 785-791.
202. Richardson, A.; Parsons, T. A mechanism for regulation of the adhesion-associated proteintyrosine kinase pp125FAK. *Nature* **1996**, *380*, 538-540, doi:10.1038/380538a0.
203. Kleinschmidt, E.G.; Schlaepfer, D.D. Focal adhesion kinase signaling in unexpected places. *Current opinion in cell biology* **2017**, *45*, 24-30, doi:<https://doi.org/10.1016/j.ceb.2017.01.003>.
204. Almeida, E.A.; Ilic, D.; Han, Q.; Hauck, C.R.; Jin, F.; Kawakatsu, H.; Schlaepfer, D.D.; Damsky, C.H. Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun NH(2)-terminal kinase. *The Journal of cell biology* **2000**, *149*, 741-754, doi:10.1083/jcb.149.3.741.
205. Mak, G.; Soria, J.-C.; Blagden, S.P.; Plummer, R.; Fleming, R.A.; Nebot, N.; Zhang, J.; Mazumdar, J.; Rogan, D.; Gazzah, A., et al. A phase Ib dose-finding, pharmacokinetic study of the focal adhesion kinase inhibitor GSK2256098 and trametinib in patients with advanced solid tumours. *British journal of cancer* **2019**, *120*, 975-981, doi:10.1038/s41416-019-0452-3.
206. Soria, J.C.; Gan, H.K.; Blagden, S.P.; Plummer, R.; Arkenau, H.T.; Ranson, M.; Evans, T.R.; Zalcman, G.; Bahleda, R.; Hollebecque, A., et al. A phase I, pharmacokinetic and pharmacodynamic study of GSK2256098, a focal adhesion kinase inhibitor, in patients with advanced solid tumors. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2016**, *27*, 2268-2274, doi:10.1093/annonc/mdw427.
207. Brown, N.F.; Williams, M.; Arkenau, H.-T.; Fleming, R.A.; Tolson, J.; Yan, L.; Zhang, J.; Singh, R.; Auger, K.R.; Lenox, L., et al. A study of the focal adhesion kinase inhibitor GSK2256098 in patients with recurrent glioblastoma with evaluation of tumor penetration of [11C]GSK2256098. *Neuro-oncology* **2018**, *20*, 1634-1642, doi:10.1093/neuonc/noy078.
208. Infante, J.R.; Camidge, D.R.; Mileskin, L.R.; Chen, E.X.; Hicks, R.J.; Rischin, D.; Fingert, H.; Pierce, K.J.; Xu, H.; Roberts, W.G., et al. Safety, pharmacokinetic, and pharmacodynamic phase I dose-escalation trial of PF-00562271, an inhibitor of focal adhesion kinase, in advanced solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2012**, *30*, 1527-1533, doi:10.1200/jco.2011.38.9346.
209. Jones, S.F.; Siu, L.L.; Bendell, J.C.; Cleary, J.M.; Razak, A.R.; Infante, J.R.; Pandya, S.S.; Bedard, P.L.; Pierce, K.J.; Houk, B., et al. A phase I study of VS-6063, a second-generation focal adhesion kinase inhibitor, in patients with advanced solid tumors. *Investigational new drugs* **2015**, *33*, 1100-1107, doi:10.1007/s10637-015-0282-y.

210. Patel, M.R.; Infante, J.R.; Moore, K.N.; Keegan, M.; Poli, A.; Padval, M.; Jones, S.F.; Horobin, J.; Burris, H.A. Phase 1/1b study of the FAK inhibitor defactinib (VS-6063) in combination with weekly paclitaxel for advanced ovarian cancer. *Journal of Clinical Oncology* **2014**, *32*, 5521-5521, doi:10.1200/jco.2014.32.15_suppl.5521.
211. Shimizu, T.; Fukuoka, K.; Takeda, M.; Iwasa, T.; Yoshida, T.; Horobin, J.; Keegan, M.; Vaickus, L.; Chavan, A.; Padval, M., et al. A first-in-Asian phase 1 study to evaluate safety, pharmacokinetics and clinical activity of VS-6063, a focal adhesion kinase (FAK) inhibitor in Japanese patients with advanced solid tumors. *Cancer chemotherapy and pharmacology* **2016**, *77*, 997-1003, doi:10.1007/s00280-016-3010-1.
212. Doi, T.; Yang, J.C.; Shitara, K.; Naito, Y.; Cheng, A.L.; Sarashina, A.; Pronk, L.C.; Takeuchi, Y.; Lin, C.C. Phase I Study of the Focal Adhesion Kinase Inhibitor BI 853520 in Japanese and Taiwanese Patients with Advanced or Metastatic Solid Tumors. *Target Oncol* **2019**, *14*, 57-65, doi:10.1007/s11523-019-00620-0.
213. de Jonge, M.J.A.; Steeghs, N.; Lolkema, M.P.; Hotte, S.J.; Hirte, H.W.; van der Biessen, D.A.J.; Abdul Razak, A.R.; De Vos, F.; Verheijen, R.B.; Schnell, D., et al. Phase I Study of BI 853520, an Inhibitor of Focal Adhesion Kinase, in Patients with Advanced or Metastatic Nonhematologic Malignancies. *Target Oncol* **2019**, *14*, 43-55, doi:10.1007/s11523-018-00617-1.
214. Verheijen, R.B.; van der Biessen, D.A.J.; Hotte, S.J.; Siu, L.L.; Spreafico, A.; de Jonge, M.J.A.; Pronk, L.C.; De Vos, F.; Schnell, D.; Hirte, H.W., et al. Randomized, Open-Label, Crossover Studies Evaluating the Effect of Food and Liquid Formulation on the Pharmacokinetics of the Novel Focal Adhesion Kinase (FAK) Inhibitor BI 853520. *Target Oncol* **2019**, *14*, 67-74, doi:10.1007/s11523-018-00618-0.
215. Aung, K.L.; McWhirter, E.; Welch, S.; Wang, L.; Lovell, S.; Stayner, L.-A.; Ali, S.; Malpage, A.; Makepeace, B.; Ramachandran, M., et al. A phase II trial of GSK2256098 and trametinib in patients with advanced pancreatic ductal adenocarcinoma (PDAC) (MOBILITY-002 Trial, NCT02428270). *Journal of Clinical Oncology* **2018**, *36*, 409-409, doi:10.1200/JCO.2018.36.4_suppl.409.
216. Fennell, D.A.; Baas, P.; Taylor, P.; Nowak, A.K.; Gilligan, D.; Nakano, T.; Pachter, J.A.; Weaver, D.T.; Scherpereel, A.; Pavlakis, N., et al. Maintenance Defactinib Versus Placebo After First-Line Chemotherapy in Patients With Merlin-Stratified Pleural Mesothelioma: COMMAND—A Double-Blind, Randomized, Phase II Study. *Journal of Clinical Oncology* **2019**, *37*, 790-798, doi:10.1200/jco.2018.79.0543.
217. Bueno, R.; Gill, R.R.; Lizotte, P.H.; Sprott, K.; Jackman, D.M.; Barlow, J.; Sharma, S.; Yeap, B.Y.; Chirieac, L.R.; Lebenthal, A., et al. Effect of FAK inhibitor defactinib on tumor immune changes and tumor reductions in a phase II window of opportunity study in malignant pleural mesothelioma (MPM). *Journal of Clinical Oncology* **2017**, *35*, 8555-8555, doi:10.1200/JCO.2017.35.15_suppl.8555.

218. Liu, T.-J.; LaFortune, T.; Honda, T.; Ohmori, O.; Hatakeyama, S.; Meyer, T.; Jackson, D.; de Groot, J.; Yung, W.K.A. Inhibition of both focal adhesion kinase and insulin-like growth factor-I receptor kinase suppresses glioma proliferation *in vitro* and *in vivo*. *Molecular cancer therapeutics* **2007**, *6*, 1357-1367, doi:10.1158/1535-7163.mct-06-0476.
219. Slack-Davis, J.K.; Martin, K.H.; Tilghman, R.W.; Iwanicki, M.; Ung, E.J.; Autry, C.; Luzzio, M.J.; Cooper, B.; Kath, J.C.; Roberts, W.G., et al. Cellular Characterization of a Novel Focal Adhesion Kinase Inhibitor. *Journal of Biological Chemistry* **2007**, *282*, 14845-14852, doi:10.1074/jbc.M606695200.
220. Auger, K.R.; Smitheman, K.N.; Korenchuk, S.; McHugh, C.; Kruger, R.; Van Aller, G.S.; Smallwood, A.; Gontarek, R.R.; Faitg, T.; Johnson, N. 387 The Focal Adhesion Kinase Inhibitor GSK2256098: a Potent and Selective Inhibitor for the Treatment of Cancer. *European Journal of Cancer* **2012**, *48*, 118, doi:10.1016/S0959-8049(12)72185-8.
221. Weis, S.M.; Lim, S.T.; Lutu-Fuga, K.M.; Barnes, L.A.; Chen, X.L.; Gothert, J.R.; Shen, T.L.; Guan, J.L.; Schlaepfer, D.D.; Cheresch, D.A. Compensatory role for Pyk2 during angiogenesis in adult mice lacking endothelial cell FAK. *The Journal of cell biology* **2008**, *181*, 43-50, doi:10.1083/jcb.200710038.
222. Walsh, C.; Tanjoni, I.; Uryu, S.; Tomar, A.; Nam, J.O.; Luo, H.; Phillips, A.; Patel, N.; Kwok, C.; McMahon, G., et al. Oral delivery of PND-1186 FAK inhibitor decreases tumor growth and spontaneous breast to lung metastasis in pre-clinical models. *Cancer biology & therapy* **2010**, *9*, 778-790.
223. Roberts, W.G.; Ung, E.; Whalen, P.; Cooper, B.; Hulford, C.; Autry, C.; Richter, D.; Emerson, E.; Lin, J.; Kath, J., et al. Antitumor Activity and Pharmacology of a Selective Focal Adhesion Kinase Inhibitor, PF-562,271. *Cancer research* **2008**, *68*, 1935-1944, doi:10.1158/0008-5472.can-07-5155.
224. Kang, Y.; Hu, W.; Ivan, C.; Dalton, H.J.; Miyake, T.; Pecot, C.V.; Zand, B.; Liu, T.; Huang, J.; Jennings, N.B., et al. Role of focal adhesion kinase in regulating YB-1-mediated paclitaxel resistance in ovarian cancer. *J Natl Cancer Inst* **2013**, *105*, 1485-1495, doi:10.1093/jnci/djt210.
225. Heinrich, T.; Seenisamy, J.; Emmanuvel, L.; Kulkarni, S.S.; Bomke, J.; Rohdich, F.; Greiner, H.; Esdar, C.; Krier, M.; Grädler, U., et al. Fragment-Based Discovery of New Highly Substituted 1H-Pyrrolo[2,3-b]- and 3H-Imidazolo[4,5-b]-Pyridines as Focal Adhesion Kinase Inhibitors. *Journal of medicinal chemistry* **2013**, *56*, 1160-1170, doi:10.1021/jm3016014.
226. Kurenova, E.; Liao, J.; He, D.H.; Hunt, D.; Yemma, M.; Bshara, W.; Seshadri, M.; Cance, W.G. The FAK scaffold inhibitor C4 disrupts FAK-VEGFR-3 signaling and inhibits pancreatic cancer growth. *Oncotarget* **2013**, *4*, 1632-1646, doi:10.18632/oncotarget.1365.
227. Kurenova, E.V.; Hunt, D.L.; He, D.; Magis, A.T.; Ostrov, D.A.; Cance, W.G. Small molecule chloropyramine hydrochloride (C4) targets the binding site of focal

adhesion kinase and vascular endothelial growth factor receptor 3 and suppresses breast cancer growth in vivo. *Journal of medicinal chemistry* **2009**, 52, 4716-4724, doi:10.1021/jm900159g.

228. Stewart, J.E.; Ma, X.; Megison, M.; Nabers, H.; Cance, W.G.; Kurenova, E.V.; Beierle, E.A. Inhibition of FAK and VEGFR-3 binding decreases tumorigenicity in neuroblastoma. *Molecular carcinogenesis* **2015**, 54, 9-23, doi:10.1002/mc.22070.

229. Golubovskaya, V.M.; Ho, B.; Zheng, M.; Magis, A.; Ostrov, D.; Morrison, C.; Cance, W.G. Disruption of focal adhesion kinase and p53 interaction with small molecule compound R2 reactivated p53 and blocked tumor growth. *BMC cancer* **2013**, 13, 342, doi:10.1186/1471-2407-13-342.

230. Ho, B.; Olson, G.; Figel, S.; Gelman, I.; Cance, W.G.; Golubovskaya, V.M. Nanog increases focal adhesion kinase (FAK) promoter activity and expression and directly binds to FAK protein to be phosphorylated. *J Biol Chem* **2012**, 287, 18656-18673, doi:10.1074/jbc.M111.322883.

231. Tiede, S.; Meyer-Schaller, N.; Kalathur, R.K.R.; Ivanek, R.; Fagiani, E.; Schmassmann, P.; Stillhard, P.; Hafliger, S.; Kraut, N.; Schweifer, N., et al. The FAK inhibitor BI 853520 exerts anti-tumor effects in breast cancer. *Oncogenesis* **2018**, 7, 73, doi:10.1038/s41389-018-0083-1.

232. Laszlo, V.; Valko, Z.; Ozsvar, J.; Kovacs, I.; Garay, T.; Hoda, M.A.; Klikovits, T.; Stockhammer, P.; Aigner, C.; Groger, M., et al. The FAK inhibitor BI 853520 inhibits spheroid formation and orthotopic tumor growth in malignant pleural mesothelioma. *J Mol Med (Berl)* **2019**, 97, 231-242, doi:10.1007/s00109-018-1725-7.

233. Shi, Q.; Hjelmeland, A.B.; Keir, S.T.; Song, L.; Wickman, S.; Jackson, D.; Ohmori, O.; Bigner, D.D.; Friedman, H.S.; Rich, J.N. A novel low-molecular weight inhibitor of focal adhesion kinase, TAE226, inhibits glioma growth. *Molecular carcinogenesis* **2007**, 46, 488-496, doi:10.1002/mc.20297.

234. Golubovskaya, V.M. Focal adhesion kinase as a cancer therapy target. *Anti-cancer agents in medicinal chemistry* **2010**, 10, 735-741.

235. Gabarra-Niecko, V.; Schaller, M.D.; Dunty, J.M. FAK regulates biological processes important for the pathogenesis of cancer. *Cancer metastasis reviews* **2003**, 22, 359-374.

236. van Nimwegen, M.J.; van de Water, B. Focal adhesion kinase: a potential target in cancer therapy. *Biochem Pharmacol* **2007**, 73, 597-609, doi:10.1016/j.bcp.2006.08.011.

237. Golubovskaya, V.M.; Cance, W.G. Focal adhesion kinase and p53 signaling in cancer cells. *Int Rev Cytol* **2007**, 263, 103-153, doi:10.1016/s0074-7696(07)63003-4.

238. Cance, W.G.; Harris, J.E.; Iacocca, M.V.; Roche, E.; Yang, X.; Chang, J.; Simkins, S.; Xu, L. Immunohistochemical analyses of focal adhesion kinase

expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2000**, 6, 2417-2423.

239. Ayaki, M.; Komatsu, K.; Mukai, M.; Murata, K.; Kameyama, M.; Ishiguro, S.; Miyoshi, J.; Tatsuta, M.; Nakamura, H. Reduced Expression of Focal Adhesion Kinase in Liver Metastases Compared with Matched Primary Human Colorectal Adenocarcinomas. *Clinical Cancer Research* **2001**, 7, 3106-3112.

240. Rovin, J.D.; Frierson Jr., H.F.; Ledin, W.; Parsons, J.T.; Adams, R.B. Expression of focal adhesion kinase in normal and pathologic human prostate tissues. *The Prostate* **2002**, 53, 124-132, doi:doi:10.1002/pros.10114.

241. Schneider, G.B.; Kurago, Z.; Zaharias, R.; Gruman, L.M.; Schaller, M.D.; Hendrix, M.J. Elevated focal adhesion kinase expression facilitates oral tumor cell invasion. *Cancer* **2002**, 95, 2508-2515, doi:10.1002/cncr.10992.

242. Aronsohn, M.S.; Brown, H.M.; Hauptman, G.; Kornberg, L.J. Expression of focal adhesion kinase and phosphorylated focal adhesion kinase in squamous cell carcinoma of the larynx. *The Laryngoscope* **2003**, 113, 1944-1948.

243. Lark, A.L.; Livasy, C.A.; Calvo, B.; Caskey, L.; Moore, D.T.; Yang, X.; Cance, W.G. Overexpression of Focal Adhesion Kinase in Primary Colorectal Carcinomas and Colorectal Liver Metastases. *Immunohistochemistry and Real-Time PCR Analyses* **2003**, 9, 215-222.

244. Oktay, M.H.; Oktay, K.; Hamele-Bena, D.; Buyuk, A.; Koss, L.G. Focal adhesion kinase as a marker of malignant phenotype in breast and cervical carcinomas. *Human pathology* **2003**, 34, 240-245, doi:10.1053/hupa.2003.40.

245. Theocharis, S.E.; Kouraklis, G.P.; Kakisis, J.D.; Kanelli, H.G.; Apostolakou, F.E.; Karatzas, G.M.; Koutselinis, A.S. Focal adhesion kinase expression is not a prognostic predictor in colon adenocarcinoma patients. *European Journal of Surgical Oncology* **2003**, 29, 571-574, doi:10.1016/S0748-7983(03)00120-3.

246. Itoh, S.; Maeda, T.; Shimada, M.; Aishima, S.; Shirabe, K.; Tanaka, S.; Maehara, Y. Role of expression of focal adhesion kinase in progression of hepatocellular carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2004**, 10, 2812-2817.

247. Kim, S.J.; Park, J.W.; Yoon, J.S.; Mok, J.O.; Kim, Y.J.; Park, H.K.; Kim, C.H.; Byun, D.W.; Lee, Y.J.; Jin, S.Y., et al. Increased expression of focal adhesion kinase in thyroid cancer: immunohistochemical study. *Journal of Korean medical science* **2004**, 19, 710-715, doi:10.3346/jkms.2004.19.5.710.

248. Lightfoot, H.M., Jr.; Lark, A.; Livasy, C.A.; Moore, D.T.; Cowan, D.; Dressler, L.; Craven, R.J.; Cance, W.G. Upregulation of focal adhesion kinase (FAK) expression in ductal carcinoma in situ (DCIS) is an early event in breast tumorigenesis. *Breast Cancer Res Treat* **2004**, 88, 109-116, doi:10.1007/s10549-004-1022-8.

249. Lark, A.L.; Livasy, C.A.; Dressler, L.; Moore, D.T.; Millikan, R.C.; Geradts, J.; Iacocca, M.; Cowan, D.; Little, D.; Craven, R.J., et al. High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype. *Mod Pathol* **2005**, *18*, 1289-1294, doi:10.1038/modpathol.3800424.
250. Canel, M.; Secades, P.; Rodrigo, J.-P.; Cabanillas, R.; Herrero, A.; Suarez, C.; Chiara, M.-D. Overexpression of Focal Adhesion Kinase in Head and Neck Squamous Cell Carcinoma Is Independent of *fak* Gene Copy Number. *Clinical Cancer Research* **2006**, *12*, 3272-3279, doi:10.1158/1078-0432.ccr-05-1583.
251. Rodrigo, J.P.; Dominguez, F.; Suárez, V.; Canel, M.; Secades, P.; Chiara, M.D. Focal Adhesion Kinase and E-Cadherin as Markers for Nodal Metastasis in Laryngeal Cancer. *Archives of Otolaryngology–Head & Neck Surgery* **2007**, *133*, 145-150, doi:10.1001/archotol.133.2.145.
252. Beierle, E.A.; Massoll, N.A.; Hartwich, J.; Kurenova, E.V.; Golubovskaya, V.M.; Cance, W.G.; McGrady, P.; London, W.B. Focal adhesion kinase expression in human neuroblastoma: immunohistochemical and real-time PCR analyses. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2008**, *14*, 3299-3305, doi:10.1158/1078-0432.ccr-07-1511.
253. Murata, T.; Naomoto, Y.; Yamatsuji, T.; Okawa, T.; Shirakawa, Y.; Gunduz, M.; Nobuhisa, T.; Takaoka, M.; Sirmali, M.; Nakajima, M., et al. Localization of FAK is related with colorectal carcinogenesis. *International journal of oncology* **2008**, *32*, 791-796.
254. Cai, L.; Han, J.; Zhuo, X.; Xiong, Y.; Dong, J.; Li, X. Overexpression and significance of focal adhesion kinase in hepatocellular carcinoma and its relationship with HBV infection. *Medical oncology (Northwood, London, England)* **2009**, *26*, 409-414, doi:10.1007/s12032-008-9137-0.
255. Golubovskaya, V.M.; Conway-Dorsey, K.; Edmiston, S.N.; Tse, C.K.; Lark, A.A.; Livasy, C.A.; Moore, D.; Millikan, R.C.; Cance, W.G. FAK overexpression and p53 mutations are highly correlated in human breast cancer. *International journal of cancer. Journal international du cancer* **2009**, *125*, 1735-1738, doi:10.1002/ijc.24486.
256. Lau, G.M.; Lau, G.M.; Yu, G.L.; Gelman, I.H.; Gutowski, A.; Hangauer, D.; Fang, J.W. Expression of Src and FAK in hepatocellular carcinoma and the effect of Src inhibitors on hepatocellular carcinoma in vitro. *Digestive diseases and sciences* **2009**, *54*, 1465-1474, doi:10.1007/s10620-008-0519-0.
257. Theocharis, S.E.; Klijanienko, J.T.; Padoy, E.; Athanassiou, S.; Sastre-Garau, X.X. Focal adhesion kinase (FAK) immunocytochemical expression in breast ductal invasive carcinoma (DIC): correlation with clinicopathological parameters and tumor proliferative capacity. *Medical science monitor : international medical journal of experimental and clinical research* **2009**, *15*, Br221-226.

258. Lai, I.R.; Chu, P.Y.; Lin, H.S.; Liou, J.Y.; Jan, Y.J.; Lee, J.C.; Shen, T.L. Phosphorylation of focal adhesion kinase at Tyr397 in gastric carcinomas and its clinical significance. *The American journal of pathology* **2010**, *177*, 1629-1637, doi:10.2353/ajpath.2010.100172.
259. Park, J.H.; Lee, B.L.; Yoon, J.; Kim, J.; Kim, M.A.; Yang, H.K.; Kim, W.H. Focal adhesion kinase (FAK) gene amplification and its clinical implications in gastric cancer. *Human pathology* **2010**, *41*, 1664-1673, doi:10.1016/j.humpath.2010.06.004.
260. Yuan, Z.; Zheng, Q.; Fan, J.; Ai, K.X.; Chen, J.; Huang, X.Y. Expression and prognostic significance of focal adhesion kinase in hepatocellular carcinoma. *Journal of cancer research and clinical oncology* **2010**, *136*, 1489-1496, doi:10.1007/s00432-010-0806-y.
261. Yom, C.K.; Noh, D.Y.; Kim, W.H.; Kim, H.S. Clinical significance of high focal adhesion kinase gene copy number and overexpression in invasive breast cancer. *Breast Cancer Res Treat* **2011**, *128*, 647-655, doi:10.1007/s10549-010-1150-2.
262. Chen, Y.; Xia, X.; Wang, S.; Wu, X.; Zhang, J.; Zhou, Y.; Tan, Y.; He, S.; Qiang, F.; Li, A., et al. High FAK combined with low JWA expression: clinical prognostic and predictive role for adjuvant fluorouracil–leucovorin–oxaliplatin treatment in resectable gastric cancer patients. *Journal of gastroenterology* **2013**, *48*, 1034-1044, doi:10.1007/s00535-012-0724-7.
263. de Vicente, J.C.; Rosado, P.; Lequerica-Fernandez, P.; Allonca, E.; Villallain, L.; Hernandez-Vallejo, G. Focal adhesion kinase overexpression: correlation with lymph node metastasis and shorter survival in oral squamous cell carcinoma. *Head & neck* **2013**, *35*, 826-830, doi:10.1002/hed.23038.
264. Faingold, D.; Filho, V.B.; Fernandes, B.; Jagan, L.; de Barros, A.M., Jr.; Orellana, M.E.; Anteck, E.; Burnier, M.N., Jr. Expression of focal adhesion kinase in uveal melanoma and the effects of Hsp90 inhibition by 17-AAG. *Pathology, research and practice* **2014**, *210*, 739-745, doi:10.1016/j.prp.2014.06.023.
265. Golubovskaya, V.M.; Ylagan, L.; Miller, A.; Hughes, M.; Wilson, J.; Wang, D.; Brese, E.; Bshara, W.; Edge, S.; Morrison, C., et al. High focal adhesion kinase expression in breast carcinoma is associated with lymphovascular invasion and triple-negative phenotype. *BMC cancer* **2014**, *14*, 769, doi:10.1186/1471-2407-14-769.
266. Stone, R.L.; Baggerly, K.A.; Armaiz-Pena, G.N.; Kang, Y.; Sanguino, A.M.; Thanappapras, D.; Dalton, H.J.; Bottsford-Miller, J.; Zand, B.; Akbani, R., et al. Focal adhesion kinase: an alternative focus for anti-angiogenesis therapy in ovarian cancer. *Cancer biology & therapy* **2014**, *15*, 919-929, doi:10.4161/cbt.28882.
267. Li, M.; Hong, L.I.; Liao, M.; Guo, G. Expression and clinical significance of focal adhesion kinase and adrenomedullin in epithelial ovarian cancer. *Oncology letters* **2015**, *10*, 1003-1007, doi:10.3892/ol.2015.3278.

268. Ren, K.; Lu, X.; Yao, N.; Chen, Y.; Yang, A.; Chen, H.; Zhang, J.; Wu, S.; Shi, X.; Wang, C., et al. Focal adhesion kinase overexpression and its impact on human osteosarcoma. *Oncotarget* **2015**, *6*, 31085-31103, doi:10.18632/oncotarget.5044.
269. Gomez Del Pulgar, T.; Cebrian, A.; Fernandez-Acenero, M.J.; Borrero-Palacios, A.; Del Puerto-Nevado, L.; Martinez-Useros, J.; Marin-Arango, J.P.; Carames, C.; Vega-Bravo, R.; Rodriguez-Remirez, M., et al. Focal adhesion kinase: predictor of tumour response and risk factor for recurrence after neoadjuvant chemoradiation in rectal cancer. *Journal of cellular and molecular medicine* **2016**, *20*, 1729-1736, doi:10.1111/jcmm.12879.
270. Omura, G.; Ando, M.; Saito, Y.; Kobayashi, K.; Yoshida, M.; Ebihara, Y.; Kanaya, K.; Fujimoto, C.; Sakamoto, T.; Kondo, K., et al. Association of the upregulated expression of focal adhesion kinase with poor prognosis and tumor dissemination in hypopharyngeal cancer. *Head & neck* **2016**, *38*, 1164-1169, doi:10.1002/hed.24176.
271. Almstedt, K.; Sicking, I.; Battista, M.J.; Huangfu, S.; Heimes, A.S.; Weyer-Elberich, V.; Hasenburg, A.; Schmidt, M. Prognostic Significance of Focal Adhesion Kinase in Node-Negative Breast Cancer. *Breast care (Basel, Switzerland)* **2017**, *12*, 329-333, doi:10.1159/000477895.
272. Thanappapasr, K.; Nartthanarung, A.; Thanappapasr, D.; Jinawath, A. pFAK-Y397 overexpression as both a prognostic and a predictive biomarker for patients with metastatic osteosarcoma. *PLoS One* **2017**, *12*, e0182989, doi:10.1371/journal.pone.0182989.
273. Gu, H.J.; Zhou, B. Focal adhesion kinase promotes progression and predicts poor clinical outcomes in patients with osteosarcoma. *Oncology letters* **2018**, *15*, 6225-6232, doi:10.3892/ol.2018.8152.
274. Munguia-Calzada, P.; Fernandez-Vega, I.; Martinez-Camblor, P.; Diaz-Coto, S.; Garcia-Pedrero, J.M.; Vivanco, B.; Osuna, C.G.; Vazquez-Lopez, F.; Rodrigo, J.P.; Santos-Juanes, J. Correlation of focal adhesion kinase expression with nodal metastasis in patients with head and neck cutaneous squamous cell carcinoma. *Head & neck* **2018**, 10.1002/hed.25556, doi:10.1002/hed.25556.
275. Schmitz, K.J.; Grabellus, F.; Callies, R.; Otterbach, F.; Wohlschlaeger, J.; Levkau, B.; Kimmig, R.; Schmid, K.W.; Baba, H.A. High expression of focal adhesion kinase (p125FAK) in node-negative breast cancer is related to overexpression of HER-2/neu and activated Akt kinase but does not predict outcome. *Breast cancer research : BCR* **2005**, *7*, R194-203, doi:10.1186/bcr977.
276. Fujii, T.; Koshikawa, K.; Nomoto, S.; Okochi, O.; Kaneko, T.; Inoue, S.; Yatabe, Y.; Takeda, S.; Nakao, A. Focal adhesion kinase is overexpressed in hepatocellular carcinoma and can be served as an independent prognostic factor. *J Hepatol* **2004**, *41*, 104-111, doi:10.1016/j.jhep.2004.03.029.

277. Sun, C.K.; Ng, K.T.; Sun, B.S.; Ho, J.W.Y.; Lee, T.K.; Ng, I.; Poon, R.T.P.; Lo, C.M.; Liu, C.L.; Man, K., et al. The significance of proline-rich tyrosine kinase2 (Pyk2) on hepatocellular carcinoma progression and recurrence. *British journal of cancer* **2007**, *97*, 50, doi:10.1038/sj.bjc.6603827.
278. Jiang, H.; Hegde, S.; Knolhoff, B.L.; Zhu, Y.; Herndon, J.M.; Meyer, M.A.; Nywening, T.M.; Hawkins, W.G.; Shapiro, I.M.; Weaver, D.T., et al. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nature medicine* **2016**, *22*, 851, doi:10.1038/nm.4123
<https://www.nature.com/articles/nm.4123#supplementary-information>.
279. Database., T.C.G.A.T. Available online: <http://www.cbioportal.org/> (accessed on 20 May 2019).
280. Agochiya, M.; Brunton, V.G.; Owens, D.W.; Parkinson, E.K.; Paraskeva, C.; Keith, W.N.; Frame, M.C. Increased dosage and amplification of the focal adhesion kinase gene in human cancer cells. *Oncogene* **1999**, *18*, 5646-5653, doi:10.1038/sj.onc.1202957.
281. Zhou, B.; Wang, G.-Z.; Zhou, G.-B.; Wen, Z.-S.; Zhou, Y.-C.; Huang, Y.-C.; Chen, Y. Somatic Mutations and Splicing Variants of Focal Adhesion Kinase in Non-Small Cell Lung Cancer. *JNCI: Journal of the National Cancer Institute* **2017**, *110*, 195-204, doi:10.1093/jnci/djx157.
282. Zeng, X.Q.; Li, N.; Ma, L.L.; Tseng, Y.J.; Zhao, N.Q.; Chen, S.Y. Prognostic Value of Focal Adhesion Kinase (FAK) in Human Solid Carcinomas: A Meta-Analysis. *PLoS One* **2016**, *11*, e0162666, doi:10.1371/journal.pone.0162666.
283. Golubovskaya, V.; Kaur, A.; Cance, W. Cloning and characterization of the promoter region of human focal adhesion kinase gene: nuclear factor kappa B and p53 binding sites. *Biochimica et biophysica acta* **2004**, *1678*, 111-125, doi:10.1016/j.bbaexp.2004.03.002.
284. Cheng, N.; Li, Y.; Han, Z.G. Argonaute2 promotes tumor metastasis by way of up-regulating focal adhesion kinase expression in hepatocellular carcinoma. *Hepatology* **2013**, *57*, 1906-1918, doi:10.1002/hep.26202.
285. Golubovskaya, V.M. FAK and Nanog cross talk with p53 in cancer stem cells. *Anti-cancer agents in medicinal chemistry* **2013**, *13*, 576-580.
286. Beviglia, L.; Kramer, R.H. HGF induces FAK activation and integrin-mediated adhesion in MTLn3 breast carcinoma cells. *International journal of cancer. Journal international du cancer* **1999**, *83*, 640-649.
287. Rodríguez-Fernández, J.L.; Rozengurt, E. Bombesin, Vasopressin, Lysophosphatidic Acid, and Sphingosylphosphorylcholine Induce Focal Adhesion Kinase Activation in Intact Swiss 3T3 Cells. *Journal of Biological Chemistry* **1998**, *273*, 19321-19328, doi:10.1074/jbc.273.30.19321.

288. Leyton, J.; Garcia-Marin, L.J.; Tapia, J.A.; Jensen, R.T.; Moody, T.W. Bombesin and gastrin releasing peptide increase tyrosine phosphorylation of focal adhesion kinase and paxillin in non-small cell lung cancer cells. *Cancer letters* **2001**, *162*, 87-95.
289. Damstrup, L.; Rygaard, K.; Spang-Thomsen, M.; Skovgaard Poulsen, H. Expression of transforming growth factor beta (TGF beta) receptors and expression of TGF beta 1, TGF beta 2 and TGF beta 3 in human small cell lung cancer cell lines. *British journal of cancer* **1993**, *67*, 1015-1021.
290. Ma, P.C.; Tretiakova, M.S.; Nallasura, V.; Jagadeeswaran, R.; Husain, A.N.; Salgia, R. Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. *British journal of cancer* **2007**, *97*, 368-377, doi:10.1038/sj.bjc.6603884.
291. Zhang, L.; Yu, H.; Badzio, A.; Boyle, T.A.; Schildhaus, H.-U.; Lu, X.; Dziadziuszko, R.; Jassem, J.; Varella-Garcia, M.; Heasley, L.E., et al. Fibroblast Growth Factor Receptor 1 and Related Ligands in Small-Cell Lung Cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* **2015**, *10*, 1083-1090, doi:10.1097/JTO.0000000000000562.
292. Thomas, A.; Lee, J.H.; Abdullaev, Z.; Park, K.S.; Pineda, M.; Saidkhodjaeva, L.; Miettinen, M.; Wang, Y.; Pack, S.D.; Giaccone, G. Characterization of fibroblast growth factor receptor 1 in small-cell lung cancer. *J Thorac Oncol* **2014**, *9*, 567-571, doi:10.1097/jto.0000000000000089.
293. Tallett, A.; Chilvers, E.R.; MacKinnon, A.C.; Haslett, C.; Sethi, T. Neuropeptides stimulate tyrosine phosphorylation and tyrosine kinase activity in small cell lung cancer cell lines. *Peptides* **1996**, *17*, 665-673, doi:10.1016/0196-9781(96)00055-1.
294. Frisch, S.M.; Vuori, K.; Ruoslahti, E.; Chan-Hui, P.Y. Control of adhesion-dependent cell survival by focal adhesion kinase. *The Journal of cell biology* **1996**, *134*, 793-799, doi:10.1083/jcb.134.3.793.
295. Kurenova, E.; Xu, L.H.; Yang, X.; Baldwin, A.S., Jr.; Craven, R.J.; Hanks, S.K.; Liu, Z.G.; Cance, W.G. Focal adhesion kinase suppresses apoptosis by binding to the death domain of receptor-interacting protein. *Molecular and cellular biology* **2004**, *24*, 4361-4371, doi:10.1128/mcb.24.10.4361-4371.2004.
296. Schlaepfer, D.D.; Hanks, S.K.; Hunter, T.; van der Geer, P. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* **1994**, *372*, 786-791, doi:10.1038/372786a0.
297. Oktay, M.; Wary, K.K.; Dans, M.; Birge, R.B.; Giancotti, F.G. Integrin-mediated activation of focal adhesion kinase is required for signaling to Jun NH2-terminal kinase and progression through the G1 phase of the cell cycle. *The Journal of cell biology* **1999**, *145*, 1461-1469, doi:10.1083/jcb.145.7.1461.

298. Zhao, J.; Bian, Z.C.; Yee, K.; Chen, B.P.; Chien, S.; Guan, J.L. Identification of transcription factor KLF8 as a downstream target of focal adhesion kinase in its regulation of cyclin D1 and cell cycle progression. *Mol Cell* **2003**, *11*, 1503-1515.
299. Zhao, J.; Pestell, R.; Guan, J.L. Transcriptional activation of cyclin D1 promoter by FAK contributes to cell cycle progression. *Mol Biol Cell* **2001**, *12*, 4066-4077, doi:10.1091/mbc.12.12.4066.
300. Zhao, J.H.; Reiske, H.; Guan, J.L. Regulation of the cell cycle by focal adhesion kinase. *The Journal of cell biology* **1998**, *143*, 1997-2008, doi:10.1083/jcb.143.7.1997.
301. Bond, M.; Sala-Newby, G.B.; Newby, A.C. Focal adhesion kinase (FAK)-dependent regulation of S-phase kinase-associated protein-2 (Skp-2) stability. A novel mechanism regulating smooth muscle cell proliferation. *The Journal of biological chemistry* **2004**, *279*, 37304-37310, doi:10.1074/jbc.M404307200.
302. Bryant, P.; Zheng, Q.; Pumiglia, K. Focal adhesion kinase controls cellular levels of p27/Kip1 and p21/Cip1 through Skp2-dependent and -independent mechanisms. *Molecular and cellular biology* **2006**, *26*, 4201-4213, doi:10.1128/mcb.01612-05.
303. Carrano, A.C.; Eytan, E.; Hershko, A.; Pagano, M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nature cell biology* **1999**, *1*, 193-199.
304. Ding, Q.; Grammer, J.R.; Nelson, M.A.; Guan, J.L.; Stewart, J.E., Jr.; Gladson, C.L. p27Kip1 and cyclin D1 are necessary for focal adhesion kinase regulation of cell cycle progression in glioblastoma cells propagated in vitro and in vivo in the scid mouse brain. *The Journal of biological chemistry* **2005**, *280*, 6802-6815, doi:10.1074/jbc.M409180200.
305. Walker, J.L.; Fournier, A.K.; Assoian, R.K. Regulation of growth factor signaling and cell cycle progression by cell adhesion and adhesion-dependent changes in cellular tension. *Cytokine Growth Factor Rev* **2005**, *16*, 395-405, doi:10.1016/j.cytogfr.2005.03.003.
306. Cance, W.G.; Golubovskaya, V.M. Focal adhesion kinase versus p53: apoptosis or survival? *Science signaling* **2008**, *1*, pe22-pe22, doi:10.1126/stke.120pe22.
307. Lim, S.-T.; Mikolon, D.; Stupack, D.; Schlaepfer, D. FERM control of FAK function: Implications for cancer therapy. *Cell cycle* **2008**, *7*, 2306-2314.
308. Haskell, H.; Natarajan, M.; Hecker, T.P.; Ding, Q.; Stewart, J., Jr.; Grammer, J.R.; Gladson, C.L. Focal adhesion kinase is expressed in the angiogenic blood vessels of malignant astrocytic tumors in vivo and promotes capillary tube formation of brain microvascular endothelial cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2003**, *9*, 2157-2165.

309. Hauck, C.R.; Hsia, D.A.; Ilic, D.; Schlaepfer, D.D. v-Src SH3-enhanced interaction with focal adhesion kinase at beta 1 integrin-containing invadopodia promotes cell invasion. *The Journal of biological chemistry* **2002**, *277*, 12487-12490, doi:10.1074/jbc.C100760200.
310. Hsia, D.A.; Mitra, S.K.; Hauck, C.R.; Streblow, D.N.; Nelson, J.A.; Ilic, D.; Huang, S.; Li, E.; Nemerow, G.R.; Leng, J., et al. Differential regulation of cell motility and invasion by FAK. *The Journal of cell biology* **2003**, *160*, 753-767, doi:10.1083/jcb.200212114.
311. Kornberg, L.J. Focal adhesion kinase and its potential involvement in tumor invasion and metastasis. *Head & neck* **1998**, *20*, 745-752.
312. Burridge, K.; Turner, C.E.; Romer, L.H. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *The Journal of cell biology* **1992**, *119*, 893-903, doi:10.1083/jcb.119.4.893.
313. Ridley, A.J.; Schwartz, M.A.; Burridge, K.; Firtel, R.A.; Ginsberg, M.H.; Borisy, G.; Parsons, J.T.; Horwitz, A.R. Cell migration: integrating signals from front to back. *Science* **2003**, *302*, 1704-1709, doi:10.1126/science.1092053.
314. Sheetz, M.P.; Felsenfeld, D.P.; Galbraith, C.G. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends Cell Biol* **1998**, *8*, 51-54.
315. Pelham, R.J., Jr.; Wang, Y. High resolution detection of mechanical forces exerted by locomoting fibroblasts on the substrate. *Mol Biol Cell* **1999**, *10*, 935-945, doi:10.1091/mbc.10.4.935.
316. Katsumi, A.; Orr, A.W.; Tzima, E.; Schwartz, M.A. Integrins in mechanotransduction. *The Journal of biological chemistry* **2004**, *279*, 12001-12004, doi:10.1074/jbc.R300038200.
317. Hanks, S.K.; Ryzhova, L.; Shin, N.Y.; Brabek, J. Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. *Frontiers in bioscience : a journal and virtual library* **2003**, *8*, d982-996.
318. Mitra, S.K.; Hanson, D.A.; Schlaepfer, D.D. Focal adhesion kinase: in command and control of cell motility. *Nat Rev Mol Cell Biol* **2005**, *6*, 56-68, doi:10.1038/nrm1549.
319. Parsons, J.T. Focal adhesion kinase: the first ten years. *Journal of cell science* **2003**, *116*, 1409-1416.
320. Siesser, P.M.; Hanks, S.K. The signaling and biological implications of FAK overexpression in cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2006**, *12*, 3233-3237, doi:10.1158/1078-0432.CCR-06-0456.
321. Webb, D.J.; Donais, K.; Whitmore, L.A.; Thomas, S.M.; Turner, C.E.; Parsons, J.T.; Horwitz, A.F. FAK-Src signalling through paxillin, ERK and MLCK

regulates adhesion disassembly. *Nature cell biology* **2004**, 6, 154-161, doi:10.1038/ncb1094.

322. Tilghman, R.W.; Slack-Davis, J.K.; Sergina, N.; Martin, K.H.; Iwanicki, M.; Hershey, E.D.; Beggs, H.E.; Reichardt, L.F.; Parsons, J.T. Focal adhesion kinase is required for the spatial organization of the leading edge in migrating cells. *Journal of cell science* **2005**, 118, 2613-2623, doi:10.1242/jcs.02380.

323. Klemke, R.L.; Leng, J.; Molander, R.; Brooks, P.C.; Vuori, K.; Cheresch, D.A. CAS/Crk coupling serves as a "molecular switch" for induction of cell migration. *The Journal of cell biology* **1998**, 140, 961-972, doi:10.1083/jcb.140.4.961.

324. Cary, L.A.; Chang, J.F.; Guan, J.L. Stimulation of cell migration by overexpression of focal adhesion kinase and its association with Src and Fyn. *Journal of cell science* **1996**, 109 (Pt 7), 1787-1794.

325. Cary, L.A.; Han, D.C.; Polte, T.R.; Hanks, S.K.; Guan, J.L. Identification of p130Cas as a mediator of focal adhesion kinase-promoted cell migration. *The Journal of cell biology* **1998**, 140, 211-221, doi:10.1083/jcb.140.1.211.

326. Owen, J.D.; Ruest, P.J.; Fry, D.W.; Hanks, S.K. Induced focal adhesion kinase (FAK) expression in FAK-null cells enhances cell spreading and migration requiring both auto- and activation loop phosphorylation sites and inhibits adhesion-dependent tyrosine phosphorylation of Pyk2. *Molecular and cellular biology* **1999**, 19, 4806-4818, doi:10.1128/mcb.19.7.4806.

327. Sieg, D.J.; Hauck, C.R.; Schlaepfer, D.D. Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. *Journal of cell science* **1999**, 112 (Pt 16), 2677-2691.

328. Han, D.C.; Guan, J.L. Association of focal adhesion kinase with Grb7 and its role in cell migration. *The Journal of biological chemistry* **1999**, 274, 24425-24430, doi:10.1074/jbc.274.34.24425.

329. Han, D.C.; Shen, T.L.; Guan, J.L. Role of Grb7 targeting to focal contacts and its phosphorylation by focal adhesion kinase in regulation of cell migration. *The Journal of biological chemistry* **2000**, 275, 28911-28917, doi:10.1074/jbc.M001997200.

330. Irby, R.B.; Yeatman, T.J. Increased Src activity disrupts cadherin/catenin-mediated homotypic adhesion in human colon cancer and transformed rodent cells. *Cancer research* **2002**, 62, 2669-2674.

331. Thiery, J.P.; Sleeman, J.P. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* **2006**, 7, 131-142, doi:10.1038/nrm1835.

332. Avizienyte, E.; Frame, M.C. Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. *Current opinion in cell biology* **2005**, 17, 542-547, doi:10.1016/j.ceb.2005.08.007.

333. McLean, G.W.; Carragher, N.O.; Avizienyte, E.; Evans, J.; Brunton, V.G.; Frame, M.C. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. *Nature reviews. Cancer* **2005**, *5*, 505-515, doi:10.1038/nrc1647.
334. Lu, W.; Kang, Y. Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. *Dev Cell* **2019**, *49*, 361-374, doi:10.1016/j.devcel.2019.04.010.
335. Canadas, I.; Taus, A.; Gonzalez, I.; Villanueva, X.; Gimeno, J.; Pijuan, L.; Domine, M.; Sanchez-Font, A.; Vollmer, I.; Menendez, S., et al. High circulating hepatocyte growth factor levels associate with epithelial to mesenchymal transition and poor outcome in small cell lung cancer patients. *Oncotarget* **2014**, *5*, 5246-5256, doi:10.18632/oncotarget.2124.
336. Ito, T.; Kudoh, S.; Ichimura, T.; Fujino, K.; Hassan, W.A.; Udaka, N. Small cell lung cancer, an epithelial to mesenchymal transition (EMT)-like cancer: significance of inactive Notch signaling and expression of achaete-scute complex homologue 1. *Hum Cell* **2017**, *30*, 1-10, doi:10.1007/s13577-016-0149-3.
337. Zhao, L.; Li, J.; Liu, Y.; Zhou, W.; Shan, Y.; Fan, X.; Zhou, X.; Shan, B.; Song, Y.; Zhan, Q. Flotillin1 promotes EMT of human small cell lung cancer via TGF-beta signaling pathway. *Cancer Biol Med* **2018**, *15*, 400-414, doi:10.20892/j.issn.2095-3941.2018.0053.
338. Cicchini, C.; Laudadio, I.; Citarella, F.; Corazzari, M.; Steindler, C.; Conigliaro, A.; Fantoni, A.; Amicone, L.; Tripodi, M. TGFbeta-induced EMT requires focal adhesion kinase (FAK) signaling. *Exp Cell Res* **2008**, *314*, 143-152, doi:10.1016/j.yexcr.2007.09.005.
339. Fan, H.; Zhao, X.; Sun, S.; Luo, M.; Guan, J.L. Function of focal adhesion kinase scaffolding to mediate endophilin A2 phosphorylation promotes epithelial-mesenchymal transition and mammary cancer stem cell activities in vivo. *J Biol Chem* **2013**, *288*, 3322-3333, doi:10.1074/jbc.M112.420497.
340. Li, X.Y.; Zhou, X.; Rowe, R.G.; Hu, Y.; Schlaepfer, D.D.; Ilic, D.; Dressler, G.; Park, A.; Guan, J.L.; Weiss, S.J. Snail1 controls epithelial-mesenchymal lineage commitment in focal adhesion kinase-null embryonic cells. *J Cell Biol* **2011**, *195*, 729-738, doi:10.1083/jcb.201105103.
341. Wang, X.; Urvalek, A.M.; Liu, J.; Zhao, J. Activation of KLF8 transcription by focal adhesion kinase in human ovarian epithelial and cancer cells. *J Biol Chem* **2008**, *283*, 13934-13942, doi:10.1074/jbc.M709300200.
342. Wang, X.; Zheng, M.; Liu, G.; Xia, W.; McKeown-Longo, P.J.; Hung, M.C.; Zhao, J. Kruppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion. *Cancer Res* **2007**, *67*, 7184-7193, doi:10.1158/0008-5472.CAN-06-4729.
343. Battle. The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. **2000**.

344. Cano, A.; Pérez-Moreno, M.A.; Rodrigo, I.; Locascio, A.; Blanco, M.J.; del Barrio, M.G.; Portillo, F.; Nieto, M.A. The transcription factor Snail controls epithelial–mesenchymal transitions by repressing E-cadherin expression. *Nature Cell Biology* **2000**, *2*, 76-83, doi:10.1038/35000025.
345. Liu, S.Q.; Xu, C.Y.; Wu, W.H.; Fu, Z.H.; He, S.W.; Qin, M.B.; Huang, J.A. Sphingosine kinase 1 promotes the metastasis of colorectal cancer by inducing the epithelialmesenchymal transition mediated by the FAK/AKT/MMPs axis. *Int J Oncol* **2019**, *54*, 41-52, doi:10.3892/ijo.2018.4607.
346. Xu, C.Y.; Liu, S.Q.; Qin, M.B.; Zhuge, C.F.; Qin, L.; Qin, N.; Lai, M.Y.; Huang, J.A. SphK1 modulates cell migration and EMT-related marker expression by regulating the expression of p-FAK in colorectal cancer cells. *Int J Mol Med* **2017**, *39*, 1277-1284, doi:10.3892/ijmm.2017.2921.
347. Taliaferro-Smith. FAK activation is required for IGF1R-mediated regulation of EMT, migration, and invasion in mesenchymal triple negative breast cancer cells. **2015**.
348. Wilson, C.; Nicholes, K.; Bustos, D.; Lin, E.; Song, Q.; Stephan, J.P.; Kirkpatrick, D.S.; Settleman, J. Overcoming EMT-associated resistance to anti-cancer drugs via Src/FAK pathway inhibition. *Oncotarget* **2014**, *5*, 7328-7341, doi:10.18632/oncotarget.2397.
349. Azzi, S.; Hebda, J.; GAVARD, J. Vascular Permeability and Drug Delivery in Cancers. *Frontiers in oncology* **2013**, *3*, doi:10.3389/fonc.2013.00211.
350. Kuczyński, E.A.; Vermeulen, P.B.; Pezzella, F.; Kerbel, R.S.; Reynolds, A.R. Vessel co-option in cancer. *Nature Reviews Clinical Oncology* **2019**, 10.1038/s41571-019-0181-9, doi:10.1038/s41571-019-0181-9.
351. Reymond, N.; d'Água, B.B.; Ridley, A.J. Crossing the endothelial barrier during metastasis. *Nature Reviews Cancer* **2013**, *13*, 858, doi:10.1038/nrc3628.
352. Williamson, S.C.; Metcalf, R.L.; Trapani, F.; Mohan, S.; Antonello, J.; Abbott, B.; Leong, H.S.; Chester, C.P.E.; Simms, N.; Polanski, R., et al. Vasculogenic mimicry in small cell lung cancer. **2016**, *7*, 13322, doi:10.1038/ncomms13322
<https://www.nature.com/articles/ncomms13322#supplementary-information>.
353. Yancopoulos, G.D.; Davis, S.; Gale, N.W.; Rudge, J.S.; Wiegand, S.J.; Holash, J. Vascular-specific growth factors and blood vessel formation. *Nature* **2000**, *407*, 242-248, doi:10.1038/35025215.
354. Sun, J.-M.; Lee, K.H.; Kim, B.-S.; Kim, H.-G.; Min, Y.J.; Yi, S.Y.; Yun, H.J.; Jung, S.-H.; Lee, S.-H.; Ahn, J.S., et al. Pazopanib maintenance after first-line etoposide and platinum chemotherapy in patients with extensive disease small-cell lung cancer: a multicentre, randomised, placebo-controlled Phase II study (KCSG-LU12-07). *British journal of cancer* **2018**, *118*, 648, doi:10.1038/bjc.2017.465.

355. Marcello Tiseo; Boni, L.; Ambrosio, F.; Camerini, A.; Baldini, E.; Cinieri, S.; Brighenti, M.; Zanelli, F.; Defraia, E.; Chiari, R., et al. Italian, Multicenter, Phase III, Randomized Study of Cisplatin Plus Etoposide With or Without Bevacizumab as First-Line Treatment in Extensive-Disease Small-Cell Lung Cancer: The GOIRC-AIFA FARM6PMFJM Trial. *Journal of Clinical Oncology* **2017**, 35, 1281-1287, doi:10.1200/jco.2016.69.4844.
356. Ilic, D.; Kovacic, B.; McDonagh, S.; Jin, F.; Baumbusch, C.; Gardner David, G.; Damsky Caroline, H. Focal Adhesion Kinase Is Required for Blood Vessel Morphogenesis. *Circulation Research* **2003**, 92, 300-307, doi:10.1161/01.RES.0000055016.36679.23.
357. Ueda, H.; Nagy, T.; Shen, T.-L.; Peng, X.; Guan, J.-L.; Stokol, T.; Zhou, H.; Vassalli, J.-D.; Alcaraz, A. Overexpression of focal adhesion kinase in vascular endothelial cells promotes angiogenesis in transgenic mice. *Cardiovascular research* **2004**, 64, 421-430, doi:10.1016/j.cardiores.2004.07.012.
358. Chen, X.L.; Nam, J.-O.; Jean, C.; Lawson, C.; Walsh, C.T.; Goka, E.; Lim, S.-T.; Tomar, A.; Tancioni, I.; Uryu, S., et al. VEGF-induced vascular permeability is mediated by FAK. *Developmental cell* **2012**, 22, 146-157, doi:10.1016/j.devcel.2011.11.002.
359. Jean, C.; Chen, X.L.; Nam, J.-O.; Tancioni, I.; Uryu, S.; Lawson, C.; Ward, K.K.; Walsh, C.T.; Miller, N.L.G.; Ghassemian, M., et al. Inhibition of endothelial FAK activity prevents tumor metastasis by enhancing barrier function. *The Journal of cell biology* **2014**, 204, 247-263, doi:10.1083/jcb.201307067.
360. Stokes, J.B.; Adair, S.J.; Slack-Davis, J.K.; Walters, D.M.; Tilghman, R.W.; Hershey, E.D.; Lowrey, B.; Thomas, K.S.; Bouton, A.H.; Hwang, R.F., et al. Inhibition of Focal Adhesion Kinase by PF-562,271 Inhibits the Growth and Metastasis of Pancreatic Cancer Concomitant with Altering the Tumor Microenvironment. *Molecular cancer therapeutics* **2011**, 10, 2135-2145, doi:10.1158/1535-7163.mct-11-0261.
361. Ward, K.K.; Tancioni, I.; Lawson, C.; Miller, N.L.G.; Jean, C.; Chen, X.L.; Uryu, S.; Kim, J.; Tarin, D.; Stupack, D.G., et al. Inhibition of focal adhesion kinase (FAK) activity prevents anchorage-independent ovarian carcinoma cell growth and tumor progression. *Clinical & experimental metastasis* **2013**, 30, 579-594, doi:10.1007/s10585-012-9562-5.
362. Serrels, B.; McGivern, N.; Canel, M.; Byron, A.; Johnson, S.C.; McSorley, H.J.; Quinn, N.; Taggart, D.; Von Kreigsheim, A.; Anderton, S.M., et al. IL-33 and ST2 mediate FAK-dependent antitumor immune evasion through transcriptional networks. *Science signaling* **2017**, 10, doi:10.1126/scisignal.aan8355.
363. Haemmerle, M.; Bottsford-Miller, J.; Pradeep, S.; Taylor, M.L.; Choi, H.J.; Hansen, J.M.; Dalton, H.J.; Stone, R.L.; Cho, M.S.; Nick, A.M., et al. FAK regulates platelet extravasation and tumor growth after antiangiogenic therapy withdrawal. *The Journal of clinical investigation* **2016**, 126, 1885-1896, doi:10.1172/jci85086.

364. Borsig, L. The role of platelet activation in tumor metastasis. *Expert Review of Anticancer Therapy* **2008**, *8*, 1247-1255, doi:10.1586/14737140.8.8.1247.
365. Labelle, M.; Begum, S.; Hynes, Richard O. Direct Signaling between Platelets and Cancer Cells Induces an Epithelial-Mesenchymal-Like Transition and Promotes Metastasis. *Cancer cell* **2011**, *20*, 576-590, doi:10.1016/j.ccr.2011.09.009.
366. Cho, M.S.; Bottsford-Miller, J.; Vasquez, H.G.; Stone, R.; Zand, B.; Kroll, M.H.; Sood, A.K.; Afshar-Kharghan, V. Platelets increase the proliferation of ovarian cancer cells. *Blood* **2012**, *120*, 4869-4872, doi:10.1182/blood-2012-06-438598.
367. Roos, W.P.; Thomas, A.D.; Kaina, B. DNA damage and the balance between survival and death in cancer biology. *Nature Reviews Cancer* **2015**, *16*, 20, doi:10.1038/nrc.2015.2
<https://www.nature.com/articles/nrc.2015.2#supplementary-information>.
368. Hoeijmakers, J.H.J. DNA Damage, Aging, and Cancer. *New England Journal of Medicine* **2009**, *361*, 1475-1485, doi:10.1056/NEJMra0804615.
369. Tubbs, A.; Nussenzweig, A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. *Cell* **2017**, *168*, 644-656, doi:10.1016/j.cell.2017.01.002.
370. Byers, L.A.; Wang, J.; Nilsson, M.B.; Fujimoto, J.; Saintigny, P.; Yordy, J.; Giri, U.; Peyton, M.; Fan, Y.H.; Diao, L., et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. *Cancer Discov* **2012**, *2*, 798-811, doi:10.1158/2159-8290.CD-12-0112.
371. Pietanza, M.C.; Waqar, S.N.; Krug, L.M.; Dowlati, A.; Hann, C.L.; Chiappori, A.; Owonikoko, T.K.; Woo, K.M.; Cardnell, R.J.; Fujimoto, J., et al. Randomized, Double-Blind, Phase II Study of Temozolomide in Combination With Either Veliparib or Placebo in Patients With Relapsed-Sensitive or Refractory Small-Cell Lung Cancer. *Journal of Clinical Oncology* **2018**, *36*, 2386-2394, doi:10.1200/jco.2018.77.7672.
372. Thomas, A.; Vilimas, R.; Trindade, C.; Erwin-Cohen, R.; Roper, N.; Xi, L.; Krishnasamy, V.; Levy, E.; Mammen, A.; Nichols, S., et al. Durvalumab in Combination with Olaparib in Patients with Relapsed SCLC: Results from a Phase II Study. *J Thorac Oncol* **2019**, *14*, 1447-1457, doi:10.1016/j.jtho.2019.04.026.
373. Constanzo, J.D.; Tang, K.-j.; Rindhe, S.; Melegari, M.; Liu, H.; Tang, X.; Rodriguez-Canales, J.; Wistuba, I.; Scaglioni, P.P. PIAS1-FAK Interaction Promotes the Survival and Progression of Non-Small Cell Lung Cancer. *Neoplasia* **2016**, *18*, 282-293, doi:<https://doi.org/10.1016/j.neo.2016.03.003>.
374. Tang, K.-J.; Constanzo, J.D.; Venkateswaran, N.; Melegari, M.; Ilcheva, M.; Morales, J.C.; Skoulidis, F.; Heymach, J.V.; Boothman, D.A.; Scaglioni, P.P. Focal Adhesion Kinase Regulates the DNA Damage Response and Its Inhibition

Radiosensitizes Mutant *KRAS* Lung Cancer. *Clinical Cancer Research* **2016**, 22, 5851-5863, doi:10.1158/1078-0432.ccr-15-2603.

375. Beinke, C.; Van Beuningen, D.; Cordes, N. Ionizing radiation modules of the expression and tyrosine phosphorylation of the focal adhesion-associated proteins focal adhesion kinase (FAK) and its substrates p130cas and paxillin in A549 human lung carcinoma cells in vitro. *International journal of radiation biology* **2003**, 79, 721-731.

376. Williams, K.E.; Bundred, N.J.; Landberg, G.; Clarke, R.B.; Farnie, G. Focal adhesion kinase and Wnt signaling regulate human ductal carcinoma in situ stem cell activity and response to radiotherapy. *Stem Cells* **2015**, 33, 327-341, doi:10.1002/stem.1843.

377. Tavora, B.; Reynolds, L.E.; Batista, S.; Demircioglu, F.; Fernandez, I.; Lechertier, T.; Lees, D.M.; Wong, P.P.; Alexopoulou, A.; Elia, G., et al. Endothelial-cell FAK targeting sensitizes tumours to DNA-damaging therapy. *Nature* **2014**, 514, 112-116, doi:10.1038/nature13541.

378. Cordes, N.; Frick, S.; Brunner, T.B.; Pilarsky, C.; Grutzmann, R.; Sipos, B.; Kloppel, G.; McKenna, W.G.; Bernhard, E.J. Human pancreatic tumor cells are sensitized to ionizing radiation by knockdown of caveolin-1. *Oncogene* **2007**, 26, 6851-6862, doi:10.1038/sj.onc.1210498.

379. Eke, I.; Sandfort, V.; Mischkus, A.; Baumann, M.; Cordes, N. Antiproliferative effects of EGFR tyrosine kinase inhibition and radiation-induced genotoxic injury are attenuated by adhesion to fibronectin. *Radiother Oncol* **2006**, 80, 178-184, doi:10.1016/j.radonc.2006.07.028.

380. Estrugo, D.; Fischer, A.; Hess, F.; Scherthan, H.; Belka, C.; Cordes, N. Ligand bound beta1 integrins inhibit procaspase-8 for mediating cell adhesion-mediated drug and radiation resistance in human leukemia cells. *PLoS One* **2007**, 2, e269, doi:10.1371/journal.pone.0000269.

381. Fuks, Z.; Vlodavsky, I.; Andreeff, M.; McLoughlin, M.; Haimovitz-Friedman, A. Effects of extracellular matrix on the response of endothelial cells to radiation in vitro. *Eur J Cancer* **1992**, 28A, 725-731.

382. Li, L.; Dong, X.; Peng, F.; Shen, L. Integrin beta1 regulates the invasion and radioresistance of laryngeal cancer cells by targeting CD147. *Cancer Cell Int* **2018**, 18, 80, doi:10.1186/s12935-018-0578-z.

383. Brodin, O.; Arnberg, H.; Bergh, J.; Nilsson, S. Increased radioresistance of an in vitro transformed human small cell lung cancer cell line. *Lung Cancer* **1995**, 12, 183-198.

384. Kraus, A.C.; Ferber, I.; Bachmann, S.O.; Specht, H.; Wimmel, A.; Gross, M.W.; Schlegel, J.; Suske, G.; Schuermann, M. In vitro chemo- and radio-resistance in small cell lung cancer correlates with cell adhesion and constitutive activation of AKT and MAP kinase pathways. *Oncogene* **2002**, 21, 8683-8695, doi:10.1038/sj.onc.1205939.

385. Kasahara, T.; Koguchi, E.; Funakoshi, M.; Aizu-Yokota, E.; Sonoda, Y. Antiapoptotic action of focal adhesion kinase (FAK) against ionizing radiation. *Antioxid Redox Signal* **2002**, *4*, 491-499, doi:10.1089/15230860260196290.
386. Skinner, H.D.; Giri, U.; Yang, L.; Woo, S.H.; Story, M.D.; Pickering, C.R.; Byers, L.A.; Williams, M.D.; El-Naggar, A.; Wang, J., et al. Proteomic Profiling Identifies PTK2/FAK as a Driver of Radioresistance in HPV-negative Head and Neck Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2016**, *22*, 4643-4650, doi:10.1158/1078-0432.ccr-15-2785.
387. Nguemgo Kouam, P.; Buhler, H.; Hero, T.; Adamietz, I.A. The increased adhesion of tumor cells to endothelial cells after irradiation can be reduced by FAK-inhibition. *Radiat Oncol* **2019**, *14*, 25, doi:10.1186/s13014-019-1230-3.
388. Hehlhans, S.; Lange, I.; Eke, I.; Cordes, N. 3D cell cultures of human head and neck squamous cell carcinoma cells are radiosensitized by the focal adhesion kinase inhibitor TAE226. *Radiother Oncol* **2009**, *92*, 371-378, doi:10.1016/j.radonc.2009.08.001.
389. Hehlhans, S.; Eke, I.; Cordes, N. Targeting FAK radiosensitizes 3-dimensional grown human HNSCC cells through reduced Akt1 and MEK1/2 signaling. *Int J Radiat Oncol Biol Phys* **2012**, *83*, e669-676, doi:10.1016/j.ijrobp.2012.01.065.
390. Eke, I.; Deuse, Y.; Hehlhans, S.; Gurtner, K.; Krause, M.; Baumann, M.; Shevchenko, A.; Sandfort, V.; Cordes, N. beta(1)Integrin/FAK/cortactin signaling is essential for human head and neck cancer resistance to radiotherapy. *The Journal of clinical investigation* **2012**, *122*, 1529-1540, doi:10.1172/JCI61350.
391. Wicha, M.S.; Liu, S.; Dontu, G. Cancer stem cells: an old idea--a paradigm shift. *Cancer Res* **2006**, *66*, 1883-1890; discussion 1895-1886, doi:10.1158/0008-5472.CAN-05-3153.
392. Afify, S.M.; Seno, M. Conversion of Stem Cells to Cancer Stem Cells: Undercurrent of Cancer Initiation. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11030345.
393. Suresh, R.; Ali, S.; Ahmad, A.; Philip, P.A.; Sarkar, F.H. The Role of Cancer Stem Cells in Recurrent and Drug-Resistant Lung Cancer. *Adv Exp Med Biol* **2016**, *890*, 57-74, doi:10.1007/978-3-319-24932-2_4.
394. Salcido, C.D.; Larochele, A.; Taylor, B.J.; Dunbar, C.E.; Varticovski, L. Molecular characterisation of side population cells with cancer stem cell-like characteristics in small-cell lung cancer. *Br J Cancer* **2010**, *102*, 1636-1644, doi:10.1038/sj.bjc.6605668.
395. Wang, P.; Gao, Q.; Suo, Z.; Munthe, E.; Solberg, S.; Ma, L.; Wang, M.; Westerdaal, N.A.; Kvalheim, G.; Gaudernack, G. Identification and characterization of cells with cancer stem cell properties in human primary lung cancer cell lines. *PLoS One* **2013**, *8*, e57020, doi:10.1371/journal.pone.0057020.

396. Zhang, Z.; Zhou, Y.; Qian, H.; Shao, G.; Lu, X.; Chen, Q.; Sun, X.; Chen, D.; Yin, R.; Zhu, H., et al. Stemness and inducing differentiation of small cell lung cancer NCI-H446 cells. *Cell Death Dis* **2013**, *4*, e633, doi:10.1038/cddis.2013.152.
397. Sarvi, S.; Mackinnon, A.C.; Avlonitis, N.; Bradley, M.; Rintoul, R.C.; Rassl, D.M.; Wang, W.; Forbes, S.J.; Gregory, C.D.; Sethi, T. CD133+ cancer stem-like cells in small cell lung cancer are highly tumorigenic and chemoresistant but sensitive to a novel neuropeptide antagonist. *Cancer Res* **2014**, *74*, 1554-1565, doi:10.1158/0008-5472.CAN-13-1541.
398. Qiu, X.; Wang, Z.; Li, Y.; Miao, Y.; Ren, Y.; Luan, Y. Characterization of sphere-forming cells with stem-like properties from the small cell lung cancer cell line H446. *Cancer Lett* **2012**, *323*, 161-170, doi:10.1016/j.canlet.2012.04.004.
399. Eramo, A.; Lotti, F.; Sette, G.; Piloizzi, E.; Biffoni, M.; Di Virgilio, A.; Conticello, C.; Ruco, L.; Peschle, C.; De Maria, R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* **2008**, *15*, 504-514, doi:10.1038/sj.cdd.4402283.
400. Gutova, M.; Najbauer, J.; Gevorgyan, A.; Metz, M.Z.; Weng, Y.; Shih, C.C.; Aboody, K.S. Identification of uPAR-positive chemoresistant cells in small cell lung cancer. *PLoS One* **2007**, *2*, e243, doi:10.1371/journal.pone.0000243.
401. Codony-Servat, J.; Verlicchi, A.; Rosell, R. Cancer stem cells in small cell lung cancer. *Transl Lung Cancer Res* **2016**, *5*, 16-25, doi:10.3978/j.issn.2218-6751.2016.01.01.
402. Morise, M.; Hishida, T.; Takahashi, A.; Yoshida, J.; Ohe, Y.; Nagai, K.; Ishii, G. Clinicopathological significance of cancer stem-like cell markers in high-grade neuroendocrine carcinoma of the lung. *J Cancer Res Clin Oncol* **2015**, *141*, 2121-2130, doi:10.1007/s00432-015-1985-3.
403. Sodja, E.; Rijavec, M.; Koren, A.; Sadikov, A.; Korosec, P.; Cufer, T. The prognostic value of whole blood SOX2, NANOG and OCT4 mRNA expression in advanced small-cell lung cancer. *Radiol Oncol* **2016**, *50*, 188-196, doi:10.1515/raon-2015-0027.
404. Yang, F.; Gao, Y.; Geng, J.; Qu, D.; Han, Q.; Qi, J.; Chen, G. Elevated expression of SOX2 and FGFR1 in correlation with poor prognosis in patients with small cell lung cancer. *Int J Clin Exp Pathol* **2013**, *6*, 2846-2854.
405. Kobayashi, K.; Takahashi, H.; Inoue, A.; Harada, H.; Toshimori, S.; Kobayashi, Y.; Goto, K.; Sugimoto, K.; Yano, H.; Ohnishi, T., et al. Oct-3/4 promotes migration and invasion of glioblastoma cells. *J Cell Biochem* **2012**, *113*, 508-517, doi:10.1002/jcb.23374.
406. Liu, C.; Li, Y.; Xing, Y.; Cao, B.; Yang, F.; Yang, T.; Ai, Z.; Wei, Y.; Jiang, J. The Interaction between Cancer Stem Cell Marker CD133 and Src Protein Promotes Focal Adhesion Kinase (FAK) Phosphorylation and Cell Migration. *J Biol Chem* **2016**, *291*, 15540-15550, doi:10.1074/jbc.M115.712976.

407. Begum, A.; Ewachiw, T.; Jung, C.; Huang, A.; Norberg, K.J.; Marchionni, L.; McMillan, R.; Penchev, V.; Rajeshkumar, N.V.; Maitra, A., et al. The extracellular matrix and focal adhesion kinase signaling regulate cancer stem cell function in pancreatic ductal adenocarcinoma. *PLoS One* **2017**, *12*, e0180181, doi:10.1371/journal.pone.0180181.
408. Ou, J.; Deng, J.; Wei, X.; Xie, G.; Zhou, R.; Yu, L.; Liang, H. Fibronectin extra domain A (EDA) sustains CD133(+)/CD44(+) subpopulation of colorectal cancer cells. *Stem Cell Res* **2013**, *11*, 820-833, doi:10.1016/j.scr.2013.05.009.
409. Guan, J.L. Integrin signaling through FAK in the regulation of mammary stem cells and breast cancer. *IUBMB life* **2010**, *62*, 268-276, doi:10.1002/iub.303.
410. Jang, H.-J.; Bak, Y.; Pham, T.-H.; Kwon, S.-B.; Kim, B.-Y.; Hong, J.; Yoon, D.-Y. STK899704 inhibits stemness of cancer stem cells and migration via the FAK-MEK-ERK pathway in HT29 cells. *BMB Reports* **2018**, *51*, 596-601, doi:10.5483/BMBRep.2018.51.11.180.
411. Ginestier, C.; Liu, S.; Diebel, M.E.; Korkaya, H.; Luo, M.; Brown, M.; Wicinski, J.; Cabaud, O.; Charafe-Jauffret, E.; Birnbaum, D., et al. CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. *J Clin Invest* **2010**, *120*, 485-497, doi:10.1172/JCI39397.
412. Sun, J.; Luo, Q.; Liu, L.; Yang, X.; Zhu, S.; Song, G. Salinomycin attenuates liver cancer stem cell motility by enhancing cell stiffness and increasing F-actin formation via the FAK-ERK1/2 signalling pathway. *Toxicology* **2017**, *384*, 1-10, doi:10.1016/j.tox.2017.04.006.
413. Thakur, R.; Trivedi, R.; Rastogi, N.; Singh, M.; Mishra, D.P. Inhibition of STAT3, FAK and Src mediated signaling reduces cancer stem cell load, tumorigenic potential and metastasis in breast cancer. *Sci Rep* **2015**, *5*, 10194, doi:10.1038/srep10194.
414. Blum, W.; Pecze, L.; Felley-Bosco, E.; Wu, L.; de Perrot, M.; Schwaller, B. Stem Cell Factor-Based Identification and Functional Properties of In Vitro-Selected Subpopulations of Malignant Mesothelioma Cells. *Stem Cell Reports* **2017**, *8*, 1005-1017, doi:10.1016/j.stemcr.2017.02.005.
415. Shapiro, I.M.; Kolev, V.N.; Vidal, C.M.; Kadariya, Y.; Ring, J.E.; Wright, Q.; Weaver, D.T.; Menges, C.; Padval, M.; McClatchey, A.I., et al. Merlin deficiency predicts FAK inhibitor sensitivity: a synthetic lethal relationship. *Sci Transl Med* **2014**, *6*, 237ra268, doi:10.1126/scitranslmed.3008639.
416. Luo, M.; Fan, H.; Nagy, T.; Wei, H.; Wang, C.; Liu, S.; Wicha, M.S.; Guan, J.L. Mammary epithelial-specific ablation of the focal adhesion kinase suppresses mammary tumorigenesis by affecting mammary cancer stem/progenitor cells. *Cancer Res* **2009**, *69*, 466-474, doi:10.1158/0008-5472.CAN-08-3078.
417. Kolev, V.N.; Tam, W.F.; Wright, Q.G.; McDermott, S.P.; Vidal, C.M.; Shapiro, I.M.; Xu, Q.; Wicha, M.S.; Pachter, J.A.; Weaver, D.T. Inhibition of FAK kinase

activity preferentially targets cancer stem cells. *Oncotarget* **2017**, 8, 51733-51747, doi:10.18632/oncotarget.18517.

418. Schober, M.; Fuchs, E. Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. *Proc Natl Acad Sci U S A* **2011**, 108, 10544-10549, doi:10.1073/pnas.1107807108.

419. Vishnubalaji, R.; Manikandan, M.; Fahad, M.; Hamam, R.; Alfayez, M.; Kassem, M.; Aldahmash, A.; Alajezi, N.M. Molecular profiling of ALDH1(+) colorectal cancer stem cells reveals preferential activation of MAPK, FAK, and oxidative stress pro-survival signalling pathways. *Oncotarget* **2018**, 9, 13551-13564, doi:10.18632/oncotarget.24420.

420. Luo, M.; Zhao, X.; Chen, S.; Liu, S.; Wicha, M.S.; Guan, J.L. Distinct FAK activities determine progenitor and mammary stem cell characteristics. *Cancer research* **2013**, 73, 5591-5602, doi:10.1158/0008-5472.can-13-1351.

421. Reck, M.; Mok, T.S.K.; Nishio, M.; Jotte, R.M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodríguez-Abreu, D.; Moro-Sibilot, D., et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *The Lancet Respiratory Medicine* 10.1016/S2213-2600(19)30084-0, doi:10.1016/S2213-2600(19)30084-0.

422. Gandini, S.; Massi, D.; Mandala, M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review and meta-analysis. *Crit Rev Oncol Hematol* **2016**, 100, 88-98, doi:10.1016/j.critrevonc.2016.02.001.

423. Ott, P.A.; Elez, E.; Hiet, S.; Kim, D.-W.; Morosky, A.; Saraf, S.; Piperdi, B.; Mehner, J.M. Pembrolizumab in Patients With Extensive-Stage Small-Cell Lung Cancer: Results From the Phase Ib KEYNOTE-028 Study. *Journal of Clinical Oncology* **2017**, 35, 3823-3829, doi:10.1200/JCO.2017.72.5069.

424. Wang, W.; Hodgkinson, P.; McLaren, F.; MacKinnon, A.; Wallace, W.; Howie, S.; Sethi, T. Small cell lung cancer tumour cells induce regulatory T lymphocytes, and patient survival correlates negatively with FOXP3+ cells in tumour infiltrate. *International journal of cancer. Journal international du cancer* **2012**, 131, E928-937, doi:10.1002/ijc.27613.

425. Serrels, A.; Lund, T.; Serrels, B.; Byron, A.; McPherson, Rhoanne C.; von Kriegsheim, A.; Gómez-Cuadrado, L.; Canel, M.; Muir, M.; Ring, Jennifer E., et al. Nuclear FAK Controls Chemokine Transcription, Tregs, and Evasion of Anti-tumor Immunity. *Cell* **2015**, 163, 160-173, doi:10.1016/j.cell.2015.09.001.

426. Ring, J.; Li, Y.; Shapiro, I.; Wang, Y.; Weaver, D.; Pachter, J. FAK/PYK2 inhibitors defactinib and VS-4718 enhance immune checkpoint inhibitor efficacy. *Journal for ImmunoTherapy of Cancer* **2015**, 3, doi:10.1186/2051-1426-3-s2-p354.

427. Hoelzinger, D.B.; Smith, S.E.; Mirza, N.; Dominguez, A.L.; Manrique, S.Z.; Lustgarten, J. Blockade of CCL1 inhibits T regulatory cell suppressive function enhancing tumor immunity without affecting T effector responses. *J Immunol* **2010**, *184*, 6833-6842, doi:10.4049/jimmunol.0904084.
428. Kuehnemuth, B.; Piseddu, I.; Wiedemann, G.M.; Lausker, M.; Kuhn, C.; Hofmann, S.; Schmoeckel, E.; Endres, S.; Mayr, D.; Jeschke, U., et al. CCL1 is a major regulatory T cell attracting factor in human breast cancer. *BMC Cancer* **2018**, *18*, 1278, doi:10.1186/s12885-018-5117-8.
429. Wang, X.; Lang, M.; Zhao, T.; Feng, X.; Zheng, C.; Huang, C.; Hao, J.; Dong, J.; Luo, L.; Li, X., et al. Cancer-FOXP3 directly activated CCL5 to recruit FOXP3(+)Treg cells in pancreatic ductal adenocarcinoma. *Oncogene* **2017**, *36*, 3048-3058, doi:10.1038/onc.2016.458.
430. Tan, M.C.; Goedegebuure, P.S.; Belt, B.A.; Flaherty, B.; Sankpal, N.; Gillanders, W.E.; Eberlein, T.J.; Hsieh, C.S.; Linehan, D.C. Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. *J Immunol* **2009**, *182*, 1746-1755, doi:10.4049/jimmunol.182.3.1746.
431. Aggarwal, S.; Sharma, S.C.; S, N.D. Dynamics of regulatory T cells (Tregs) in patients with oral squamous cell carcinoma. *J Surg Oncol* **2017**, *116*, 1103-1113, doi:10.1002/jso.24782.
432. Ghiringhelli, F.; Puig, P.E.; Roux, S.; Parcellier, A.; Schmitt, E.; Solary, E.; Kroemer, G.; Martin, F.; Chauffert, B.; Zitvogel, L. Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. *J Exp Med* **2005**, *202*, 919-929, doi:10.1084/jem.20050463.
433. Gabrilovich, D.I.; Ostrand-Rosenberg, S.; Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* **2012**, *12*, 253-268, doi:10.1038/nri3175.
434. Tian, T.; Gu, X.; Zhang, B.; Liu, Y.; Yuan, C.; Shao, L.; Guo, Y.; Fan, K. Increased circulating CD14(+)HLA-DR-/low myeloid-derived suppressor cells are associated with poor prognosis in patients with small-cell lung cancer. *Cancer Biomark* **2015**, *15*, 425-432, doi:10.3233/CBM-150473.
435. Iriki, T.; Ohnishi, K.; Fujiwara, Y.; Horlad, H.; Saito, Y.; Pan, C.; Ikeda, K.; Mori, T.; Suzuki, M.; Ichiyasu, H., et al. The cell-cell interaction between tumor-associated macrophages and small cell lung cancer cells is involved in tumor progression via STAT3 activation. *Lung Cancer* **2017**, *106*, 22-32, doi:10.1016/j.lungcan.2017.01.003.
436. Wendt, M.K.; Schiemann, W.P. Therapeutic targeting of the focal adhesion complex prevents oncogenic TGF-beta signaling and metastasis. *Breast Cancer Res* **2009**, *11*, R68, doi:10.1186/bcr2360.

437. Sahai, E.; Marshall, C.J. RHO–GTPases and cancer. *Nature Reviews Cancer* **2002**, *2*, 133-142, doi:10.1038/nrc725.
438. Bid, H.K.; Roberts, R.D.; Manchanda, P.K.; Houghton, P.J. RAC1: An Emerging Therapeutic Option for Targeting Cancer Angiogenesis and Metastasis. *Molecular cancer therapeutics* **2013**, *12*, 1925-1934, doi:10.1158/1535-7163.Mct-13-0164.
439. Jaffe, A.B.; Hall, A. Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol* **2005**, *21*, 247-269, doi:10.1146/annurev.cellbio.21.020604.150721.
440. Kazanietz, M.G.; Caloca, M.J. The Rac GTPase in Cancer: From Old Concepts to New Paradigms. *Cancer research* **2017**, *77*, 5445-5451, doi:10.1158/0008-5472.Can-17-1456.
441. Orgaz, J.L.; Herraiz, C.; Sanz-Moreno, V. Rho GTPases modulate malignant transformation of tumor cells. *Small GTPases* **2014**, *5*, e29019, doi:10.4161/sgtp.29019.
442. Rathinam, R. Role of Rho GTPases and their regulators in cancer progression. *Frontiers in Bioscience* **2011**, *16*, 2561, doi:10.2741/3872.
443. Rottner, K.; Hall, A.; Small, J.V. Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* **1999**, *9*, 640-648, doi:10.1016/s0960-9822(99)80286-3.
444. Price, L.S.; Leng, J.; Schwartz, M.A.; Bokoch, G.M. Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol Biol Cell* **1998**, *9*, 1863-1871, doi:10.1091/mbc.9.7.1863.
445. Chang, F.; Lemmon, C.; Lietha, D.; Eck, M.; Romer, L. Tyrosine phosphorylation of Rac1: a role in regulation of cell spreading. *PLoS One* **2011**, *6*, e28587, doi:10.1371/journal.pone.0028587.
446. Hofbauer, S.W.; Krenn, P.W.; Ganghammer, S.; Asslaber, D.; Pichler, U.; Oberascher, K.; Henschler, R.; Wallner, M.; Kerschbaum, H.; Greil, R., et al. Tiam1/Rac1 signals contribute to the proliferation and chemoresistance, but not motility, of chronic lymphocytic leukemia cells. *Blood* **2014**, *123*, 2181-2188, doi:10.1182/blood-2013-08-523563.
447. Lou, S.; Wang, P.; Yang, J.; Ma, J.; Liu, C.; Zhou, M. Prognostic and Clinicopathological Value of Rac1 in Cancer Survival: Evidence from a Meta-Analysis. *Journal of Cancer* **2018**, *9*, 2571-2579, doi:10.7150/jca.24824.
448. Porter, A.P.; Papaioannou, A.; Malliri, A. Deregulation of Rho GTPases in cancer. *Small GTPases* **2016**, *7*, 123-138, doi:10.1080/21541248.2016.1173767.
449. Andrio, E.; Lotte, R.; Hamaoui, D.; Cherfils, J.; Doye, A.; Daugaard, M.; Sorensen, P.H.; Bost, F.; Ruimy, R.; Mettouchi, A., et al. Identification of cancer-associated missense mutations in hace1 that impair cell growth control and Rac1 ubiquitylation. *Scientific reports* **2017**, *7*, 44779, doi:10.1038/srep44779.

450. Liu, L.; Wong, C.C.; Gong, B.; Yu, J. Functional significance and therapeutic implication of ring-type E3 ligases in colorectal cancer. *Oncogene* **2018**, *37*, 148-159, doi:10.1038/onc.2017.313.
451. Cooke, M.; Baker, M.J.; Kazanietz, M.G. Rac-GEF/Rac Signaling and Metastatic Dissemination in Lung Cancer. *Front Cell Dev Biol* **2020**, *8*, 118-118, doi:10.3389/fcell.2020.00118.
452. Zheng, C.-W.; Zeng, R.-J.; Xu, L.-Y.; Li, E.-M. Rho GTPases: Promising candidates for overcoming chemotherapeutic resistance. *Cancer letters* **2020**, *475*, 65-78, doi:<https://doi.org/10.1016/j.canlet.2020.01.018>.
453. Li, Q.; Qin, T.; Bi, Z.; Hong, H.; Ding, L.; Chen, J.; Wu, W.; Lin, X.; Fu, W.; Zheng, F., et al. Rac1 activates non-oxidative pentose phosphate pathway to induce chemoresistance of breast cancer. *Nature communications* **2020**, *11*, 1456-1456, doi:10.1038/s41467-020-15308-7.
454. Aboubakar Nana, F.; Hoton, D.; Ambroise, J.; Lecocq, M.; Vanderputten, M.; Sibille, Y.; Vanaudenaerde, B.; Pilette, C.; Bouzin, C.; Ocak, S. Increased Expression and Activation of FAK in Small-Cell Lung Cancer Compared to Non-Small-Cell Lung Cancer. *Cancers* **2019**, *11*, doi:10.3390/cancers11101526.
455. Langer, D.A.; Kautzman, D.; Kane, M.A. Inhibition of proliferation of human small cell lung cancer cells expressing an autocrine system for gastrin releasing peptide by antisense oligodeoxynucleotides to gastrin releasing peptide receptor. *Lung cancer* **2002**, *36*, 235-242, doi:[http://dx.doi.org/10.1016/S0169-5002\(01\)00490-1](http://dx.doi.org/10.1016/S0169-5002(01)00490-1).
456. Schlaepfer, D.D.; Hauck, C.R.; Sieg, D.J. Signaling through focal adhesion kinase. *Prog Biophys Mol Biol* **1999**, *71*, 435-478.
457. Cox, B.D.; Natarajan, M.; Stettner, M.R.; Gladson, C.L. New concepts regarding focal adhesion kinase promotion of cell migration and proliferation. *Journal of cellular biochemistry* **2006**, *99*, 35-52, doi:10.1002/jcb.20956.
458. <https://seer.cancer.gov/statfacts/html/lungb.html>. Accessed 29 July 2019.
459. Roy, S.; Ruest, P.J.; Hanks, S.K. FAK regulates tyrosine phosphorylation of CAS, paxillin, and PYK2 in cells expressing v-Src, but is not a critical determinant of v-Src transformation. *Journal of cellular biochemistry* **2002**, *84*, 377-388.
460. Aboubakar Nana, F.; Lecocq, M.; Ladjemi, M.Z.; Detry, B.; Dupasquier, S.; Feron, O.; Massion, P.P.; Sibille, Y.; Pilette, C.; Ocak, S. Therapeutic Potential of Focal Adhesion Kinase Inhibition in Small Cell Lung Cancer. *Molecular cancer therapeutics* **2019**, *18*, 17-27, doi:10.1158/1535-7163.Mct-18-0328.
461. Zhang, H.; Shao, H.; Golubovskaya, V.M.; Chen, H.; Cance, W.; Adjei, A.A.; Dy, G.K. Efficacy of focal adhesion kinase inhibition in non-small cell lung cancer with oncogenically activated MAPK pathways. *British journal of cancer* **2016**, *115*, 203, doi:10.1038/bjc.2016.190

<https://www.nature.com/articles/bjc2016190#supplementary-information> accessed on 8th February 2019.

462. Kononen, J.; Bubendorf, L.; Kallionimeni, A.; Bärnlund, M.; Schraml, P.; Leighton, S.; Torhorst, J.; Mihatsch, M.J.; Sauter, G.; Kallionimeni, O.-P. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nature medicine* **1998**, *4*, 844-847, doi:10.1038/nm0798-844.
463. Massion, P.P.; Kuo, W.-L.; Stokoe, D.; Olshen, A.B.; Treseler, P.A.; Chin, K.; Chen, C.; Polikoff, D.; Jain, A.N.; Pinkel, D., et al. Genomic Copy Number Analysis of Non-small Cell Lung Cancer Using Array Comparative Genomic Hybridization. *Implications of the Phosphatidylinositol 3-Kinase Pathway* **2002**, *62*, 3636-3640.
464. Travis, W.D.; Brambilla, E.; Nicholson, A.G.; Yatabe, Y.; Austin, J.H.M.; Beasley, M.B.; Chirieac, L.R.; Dacic, S.; Duhig, E.; Flieder, D.B., et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. *J Thorac Oncol* **2015**, *10*, 1243-1260, doi:10.1097/jto.0000000000000630.
465. Serrels, A.; McLeod, K.; Canel, M.; Kinnaird, A.; Graham, K.; Frame, M.C.; Brunton, V.G. The role of focal adhesion kinase catalytic activity on the proliferation and migration of squamous cell carcinoma cells. *International Journal of Cancer* **2012**, *131*, 287-297, doi:10.1002/ijc.26351.
466. Beierle, E.A.; Ma, X.; Stewart, J.; Nyberg, C.; Trujillo, A.; Cance, W.G.; Golubovskaya, V.M. Inhibition of focal adhesion kinase decreases tumor growth in human neuroblastoma. *Cell cycle* **2010**, *9*, 1005-1015, doi:10.4161/cc.9.5.10936.
467. Hochwald, S.N.; Nyberg, C.; Zheng, M.; Zheng, D.; Wood, C.; Massoll, N.A.; Magis, A.; Ostrov, D.; Cance, W.G.; Golubovskaya, V.M. A novel small molecule inhibitor of FAK decreases growth of human pancreatic cancer. *Cell cycle* **2009**, *8*, 2435-2443, doi:10.4161/cc.8.15.9145.
468. Nishimura, M.; Machida, K.; Imaizumi, M.; Abe, T.; Umeda, T.; Takeshima, E.; Watanabe, T.; Ohnishi, Y.; Takagi, K.; Hamaguchi, M. Tyrosine phosphorylation of 100-130 kDa proteins in lung cancer correlates with poor prognosis. *British journal of cancer* **1996**, *74*, 780-787.
469. Wang, B.; Qi, X.; Li, D.; Feng, M.; Meng, X.; Fu, S. Expression of pY397 FAK promotes the development of non-small cell lung cancer. *Oncology letters* **2016**, *11*, 979-983, doi:10.3892/ol.2015.3992.
470. Lim, S.T.; Chen, X.L.; Lim, Y.; Hanson, D.A.; Vo, T.T.; Howerton, K.; Larocque, N.; Fisher, S.J.; Schlaepfer, D.D.; Ilic, D. Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol Cell* **2008**, *29*, 9-22, doi:10.1016/j.molcel.2007.11.031.
471. Lim, S.T. Nuclear FAK: a new mode of gene regulation from cellular adhesions. *Molecules and cells* **2013**, *36*, 1-6, doi:10.1007/s10059-013-0139-1.

472. Parsons, S.J.; Parsons, J.T. Src family kinases, key regulators of signal transduction. *Oncogene* **0000**, 23, 7906-7909.
473. Ruest, P.J.; Shin, N.Y.; Polte, T.R.; Zhang, X.; Hanks, S.K. Mechanisms of CAS substrate domain tyrosine phosphorylation by FAK and Src. *Molecular and cellular biology* **2001**, 21, 7641-7652, doi:10.1128/mcb.21.22.7641-7652.2001.
474. Lee, B.Y.; Timpson, P.; Horvath, L.G.; Daly, R.J. FAK signaling in human cancer as a target for therapeutics. *Pharmacology & therapeutics* **2015**, 146, 132-149, doi:10.1016/j.pharmthera.2014.10.001.
475. Eke, I.; Cordes, N. Focal adhesion signaling and therapy resistance in cancer. *Seminars in cancer biology* **2015**, 31, 65-75, doi:10.1016/j.semcancer.2014.07.009.
476. Golubovskaya, V.M. Targeting FAK in human cancer: from finding to first clinical trials. *Frontiers in bioscience (Landmark edition)* **2014**, 19, 687-706.
477. Shanthi, E.; Krishna, M.H.; Arunesh, G.M.; Venkateswara Reddy, K.; Sooriya Kumar, J.; Viswanadhan, V.N. Focal adhesion kinase inhibitors in the treatment of metastatic cancer: a patent review. *Expert opinion on therapeutic patents* **2014**, 24, 1077-1100, doi:10.1517/13543776.2014.948845.
478. Tiscornia, G.; Singer, O.; Verma, I.M. Production and purification of lentiviral vectors. *Nat. Protocols* **2006**, 1, 241-245.
479. Bryant, P.W.; Zheng, Q.; Pumiglia, K.M. Focal adhesion kinase is a phospho-regulated repressor of Rac and proliferation in human endothelial cells. *Biology Open* **2012**, 1, 723-730, doi:10.1242/bio.20121008.
480. Gastonguay, A.; J. Berg, T.; Hauser, A.; Schuld, N.; L. Lorimer, E.; L Williams, C. The role of Rac1 in the regulation of NF- κ B activity, cell proliferation, and cell migration in non-small cell lung carcinoma; 2012; Vol. 13, pp. 647-656.
481. Valles, A.M.; Beuvin, M.; Boyer, B. Activation of Rac1 by paxillin-Crk-DOCK180 signaling complex is antagonized by Rap1 in migrating NBT-II cells. *The Journal of biological chemistry* **2004**, 279, 44490-44496, doi:10.1074/jbc.M405144200.
482. Kurio, N.; Shimo, T.; Fukazawa, T.; Takaoka, M.; Okui, T.; Hassan, N.M.; Honami, T.; Hatakeyama, S.; Ikeda, M.; Naomoto, Y., et al. Anti-tumor effect in human breast cancer by TAE226, a dual inhibitor for FAK and IGF-IR in vitro and in vivo. *Experimental cell research* **2011**, 317, 1134-1146, doi:10.1016/j.yexcr.2011.02.008.
483. Otani, H.; Yamamoto, H.; Takaoka, M.; Sakaguchi, M.; Soh, J.; Jida, M.; Ueno, T.; Kubo, T.; Asano, H.; Tsukuda, K., et al. TAE226, a Bis-Anilino Pyrimidine Compound, Inhibits the EGFR-Mutant Kinase Including T790M Mutant to Show Anti-Tumor Effect on EGFR-Mutant Non-Small Cell Lung Cancer Cells. *PLoS One* **2015**, 10, e0129838, doi:10.1371/journal.pone.0129838.

484. Wang, Z.G.; Fukazawa, T.; Nishikawa, T.; Watanabe, N.; Sakurama, K.; Motoki, T.; Takaoka, M.; Hatakeyama, S.; Omori, O.; Ohara, T., et al. TAE226, a dual inhibitor for FAK and IGF-IR, has inhibitory effects on mTOR signaling in esophageal cancer cells. *Oncology reports* **2008**, *20*, 1473-1477.
485. Golubovskaya, V.; Curtin, L.; Groman, A.; Sexton, S.; Cance, W.G. In vivo toxicity, metabolism and pharmacokinetic properties of FAK inhibitor 14 or Y15 (1, 2, 4, 5-benzenetetramine tetrahydrochloride). *Archives of toxicology* **2015**, *89*, 1095-1101, doi:10.1007/s00204-014-1290-y.
486. Parri, M.; Chiarugi, P. Rac and Rho GTPases in cancer cell motility control. *Cell communication and signaling : CCS* **2010**, *8*, 23, doi:10.1186/1478-811x-8-23.
487. Moissoglu, K.; Sachdev, S.; Gelman, I.H. Enhanced v-Src-induced oncogenic transformation in the absence of focal adhesion kinase is mediated by phosphatidylinositol 3-kinase. *Biochemical and biophysical research communications* **2005**, *330*, 673-684, doi:10.1016/j.bbrc.2005.03.025.
488. Tong, J.H.; Yeung, S.F.; Chan, A.W.H.; Chung, L.Y.; Chau, S.L.; Lung, R.W.M.; Tong, C.Y.; Chow, C.; Tin, E.K.Y.; Yu, Y.H., et al. MET Amplification and Exon 14 Splice Site Mutation Define Unique Molecular Subgroups of Non-Small Cell Lung Carcinoma with Poor Prognosis. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2016**, *22*, 3048-3056, doi:10.1158/1078-0432.CCR-15-2061.
489. Cappuzzo, F.; Marchetti, A.; Skokan, M.; Rossi, E.; Gajapathy, S.; Felicioni, L.; Del Grammastio, M.; Sciarrotta, M.G.; Buttitta, F.; Incarbone, M., et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2009**, *27*, 1667-1674, doi:10.1200/JCO.2008.19.1635.
490. Oh, D.-Y.; Bang, Y.-J. HER2-targeted therapies — a role beyond breast cancer. *Nature Reviews Clinical Oncology* **2019**, 10.1038/s41571-019-0268-3, doi:10.1038/s41571-019-0268-3.
491. Peters, S.; Stahel, R.; Bubendorf, L.; Bonomi, P.; Villegas, A.; Kowalski, D.M.; Baik, C.S.; Isla, D.; Carpeno, J.C.; Garrido, P., et al. Trastuzumab Emtansine (T-DM1) in Patients with Previously Treated HER2-Overexpressing Metastatic Non-Small Cell Lung Cancer: Efficacy, Safety, and Biomarkers. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2019**, *25*, 64-72, doi:10.1158/1078-0432.Ccr-18-1590.
492. Li, B.T.; Ross, D.S.; Aisner, D.L.; Chaft, J.E.; Hsu, M.; Kako, S.L.; Kris, M.G.; Varella-Garcia, M.; Arcila, M.E. *HER2* Amplification and *HER2* Mutation Are Distinct Molecular Targets in Lung Cancers. *Journal of Thoracic Oncology* **2016**, *11*, 414-419, doi:10.1016/j.jtho.2015.10.025.
493. Drilon, A.; Cappuzzo, F.; Ou, S.-H.I.; Camidge, D.R. Targeting MET in Lung Cancer: Will Expectations Finally Be MET? *Journal of Thoracic Oncology* **2017**, *12*, 15-26, doi:10.1016/j.jtho.2016.10.014.

494. Saigi, M.; Albuquerque-Bejar, J.J.; Sanchez-Céspedes, M. Determinants of immunological evasion and immuncheckpoint inhibition response in non-small cell lung cancer: the genetic front. *Oncogene* **2019**, *38*, 5921-5932, doi:10.1038/s41388-019-0855-x.
495. Mazieres, J.; Drilon, A.; Lusque, A.; Mhanna, L.; Cortot, A.B.; Mezquita, L.; Thai, A.A.; Masciaux, C.; Couraud, S.; Veillon, R., et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2019**, 10.1093/annonc/mdz167, doi:10.1093/annonc/mdz167.
496. Saigi, M.; Albuquerque-Bejar, J.J.; Mc Leer-Florin, A.; Pereira, C.; Pros, E.; Romero, O.A.; Baixeras, N.; Esteve-Codina, A.; Nadal, E.; Brambilla, E., et al. *MET*-Oncogenic and *JAK2*-Inactivating Alterations Are Independent Factors That Affect Regulation of PD-L1 Expression in Lung Cancer. *Clinical Cancer Research* **2018**, *24*, 4579-4587, doi:10.1158/1078-0432.Ccr-18-0267.
497. Odell, I.D.; Flavell, R.A. HER2 joins AKT to inhibit STING immunity. *Nature cell biology* **2019**, *21*, 917-918, doi:10.1038/s41556-019-0368-4.
498. Pitt, Jonathan M.; Vétizou, M.; Daillère, R.; Roberti, María P.; Yamazaki, T.; Routy, B.; Lepage, P.; Boneca, Ivo G.; Chamaillard, M.; Kroemer, G., et al. Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors. *Immunity* **2016**, *44*, 1255-1269, doi:10.1016/j.immuni.2016.06.001.
499. Molofsky, Ari B.; Savage, Adam K.; Locksley, Richard M. Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. *Immunity* **2015**, *42*, 1005-1019, doi:10.1016/j.immuni.2015.06.006.
-