PhD Candidacy Exam

On-topic proposal

October 13th, 2017

[redacted]

ADVISOR: [redacted]

Relevance of fibroblast growth factor receptor 1 and its isoforms in prostate cancer bone metastases

Outline

- Background
- Goal and Hypothesis
- Specific Aims: approach
    - Experimental Design
    - Expected results
    - Potential pitfalls and alternative approaches
- Conclusive statement

Background

Prostate Cancer (PCa)

Estimated New Cases

Males

Prostate	161,360	19%
Lung & bronchus	116,990	14%
Colon & rectum	71,420	9%
Urinary bladder	60,490	7%
Melanoma of the skin	52,170	6%
Kidney & renal pelvis	40,610	5%
Non-Hodgkin lymphoma	40,080	5%
Leukemia	36,290	4%
Oral cavity & pharynx	35,720	4%
Liver & intrahepatic bile duct	29,200	3%
All Sites	**836,150**	**100%**

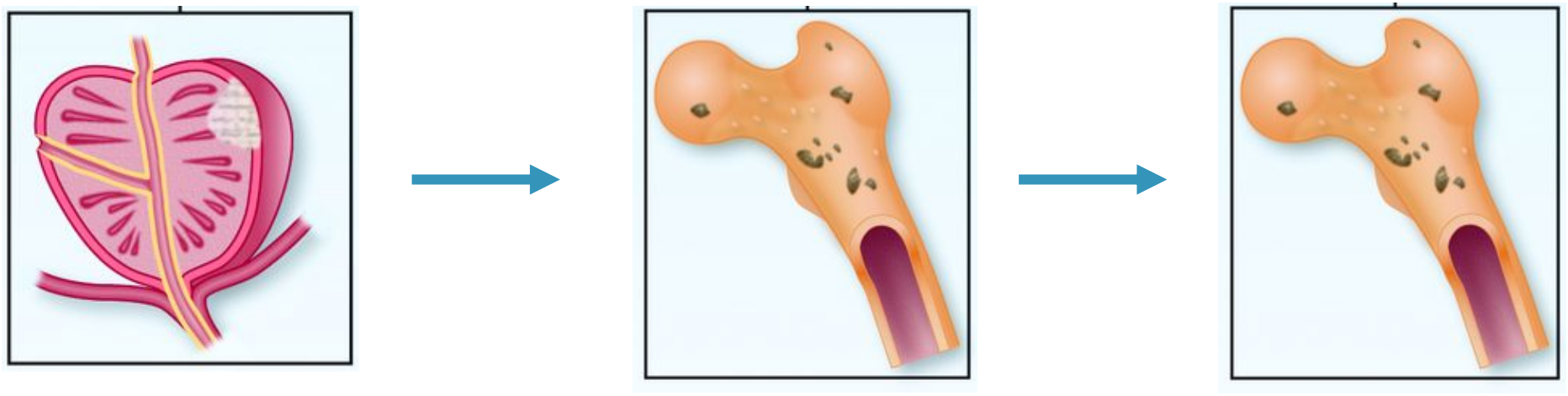
Estimated Deaths

Males

Lung & bronchus	84,590	27%
Colon & rectum	27,150	9%
Prostate	26,730	8%
Pancreas	22,300	7%
Liver & intrahepatic bile duct	19,610	6%
Leukemia	14,300	4%
Esophagus	12,720	4%
Urinary bladder	12,240	4%
Non-Hodgkin lymphoma	11,450	4%
Brain & other nervous system	9,620	3%
All Sites	**318,420**	**100%**

Siegel et al Cancer J Clin 2017

Advanced PCa



Osteoblastic

Androgen dependent

Castrate Resistant

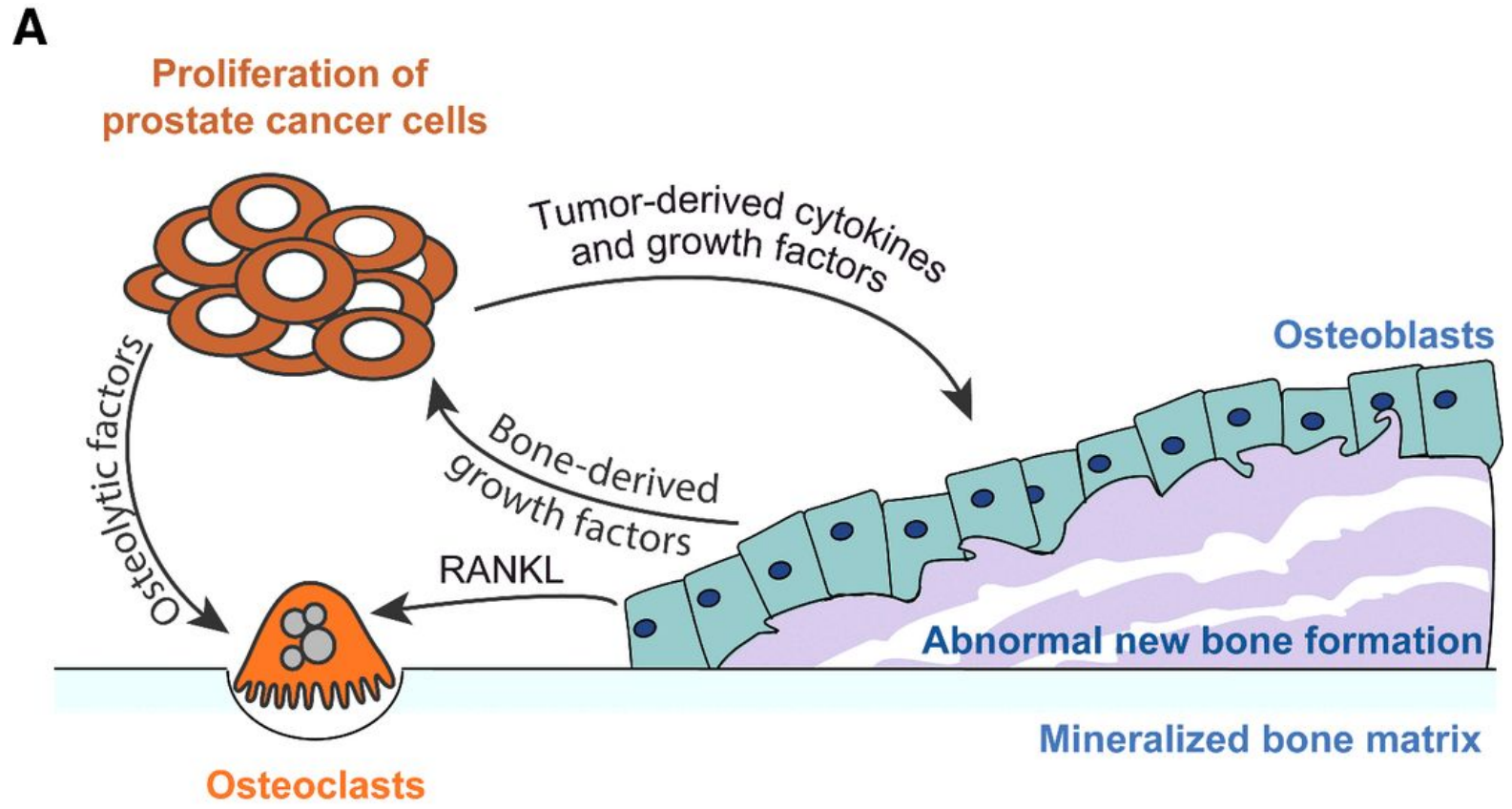
Androgen deprivation therapy

Currently no curative therapy

Clinical challenge of PCa

Adapted from Logothetis et al Cancer Discov 2013

The Vicious Cycle of Bone Metastasis

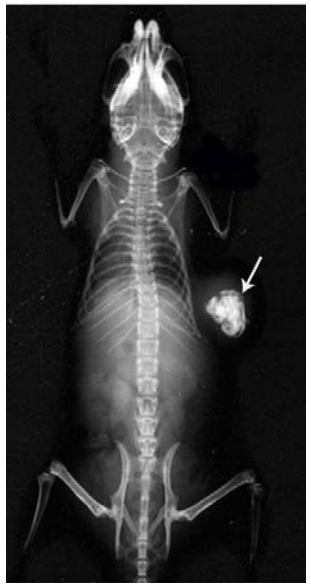


Adapted from Suominen et al Clin Cancer Res 2017

Fibroblast growth factor (FGF) axis in PCa Bone Metastases

Bone metastasis-derived xenograft MDA PCa 118b

X-ray



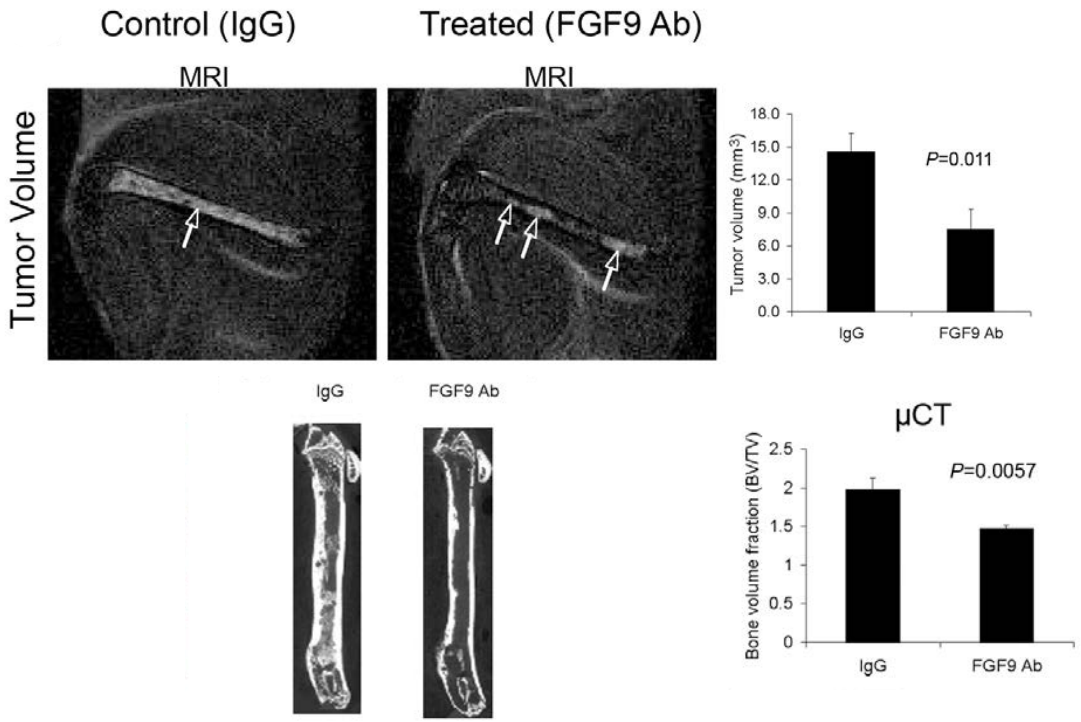
Ectopic bone formation

↓

Gene array analysis

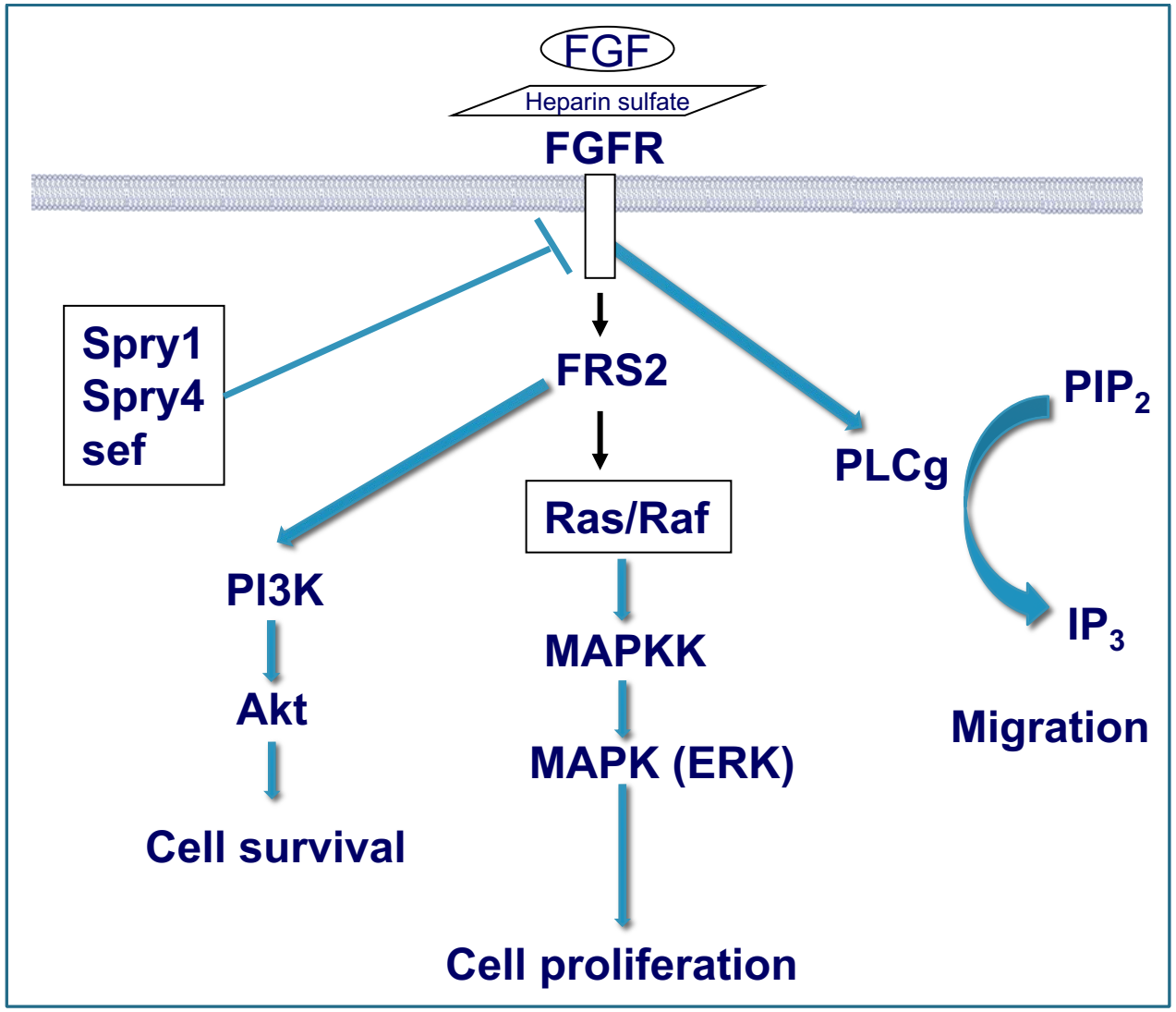
↓

FGF9



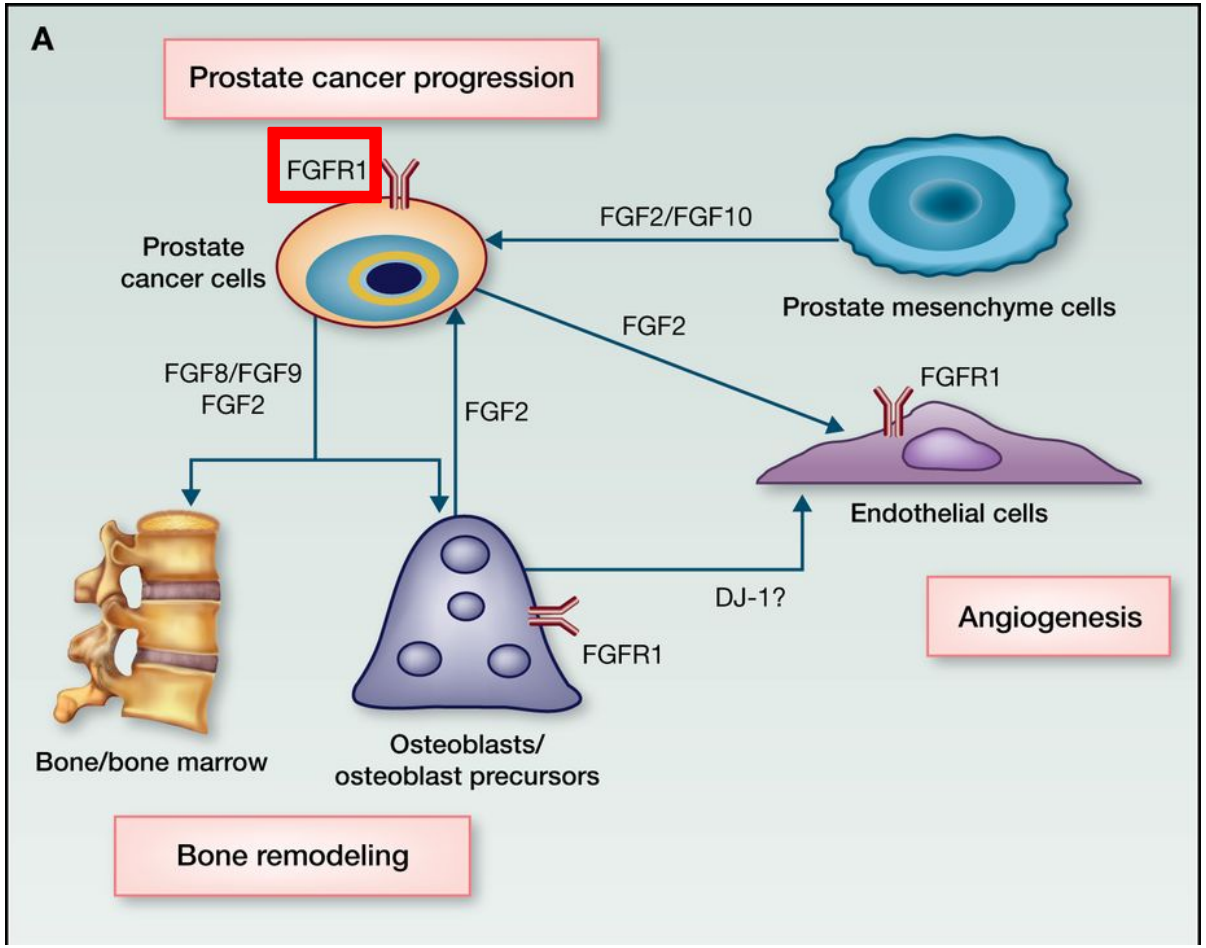
Li et al J Clin Invest 2008

FGF axis signaling and functions



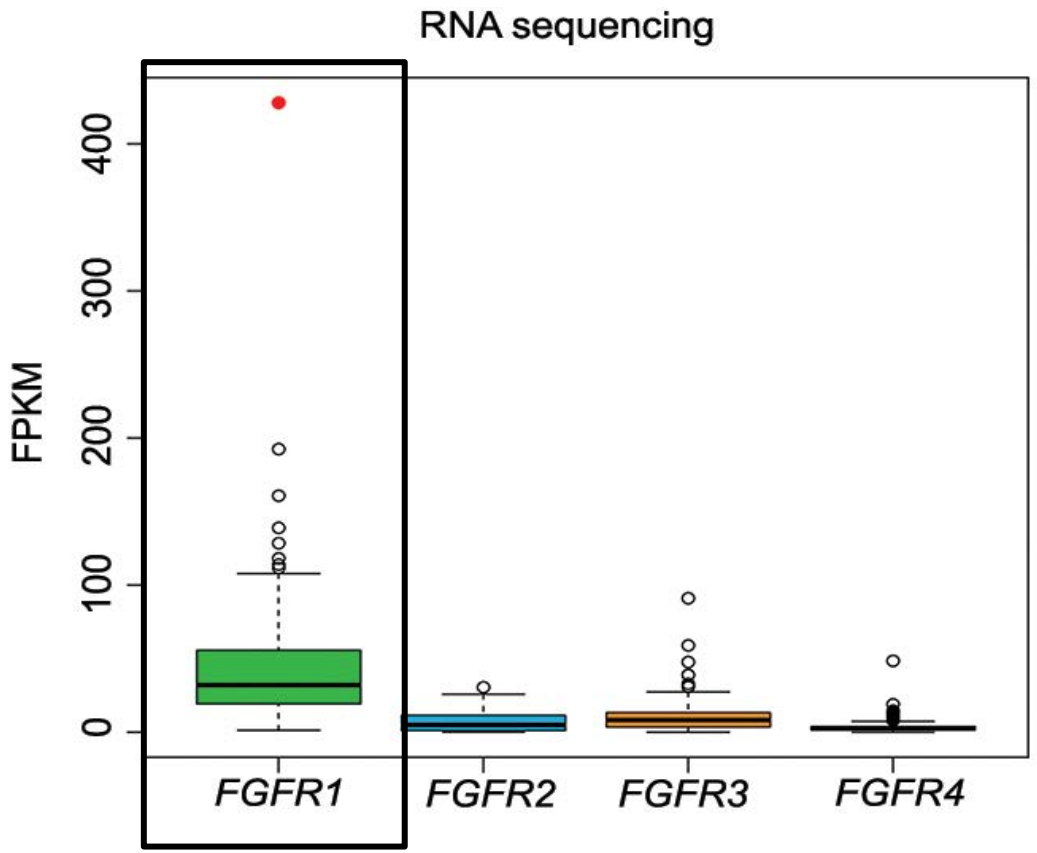
Adapted from Teven et al Genes Dis 2014

FGF signaling in PCa- stroma interaction



Corn et al Clin Cancer Res 2013

FGFRs in human PCa



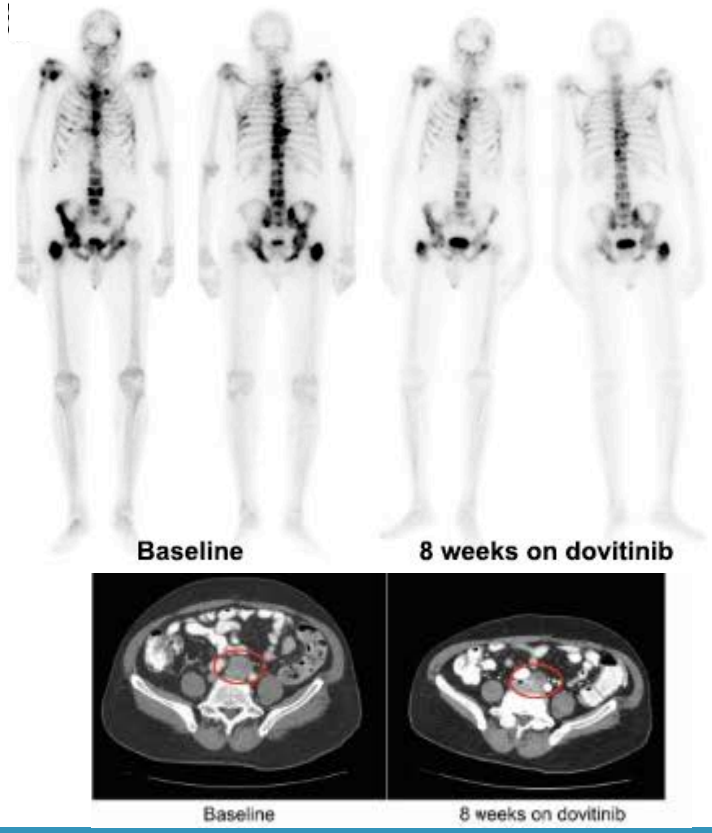
Wan et al Sci Transl Med 2014

FGFR as therapeutic target

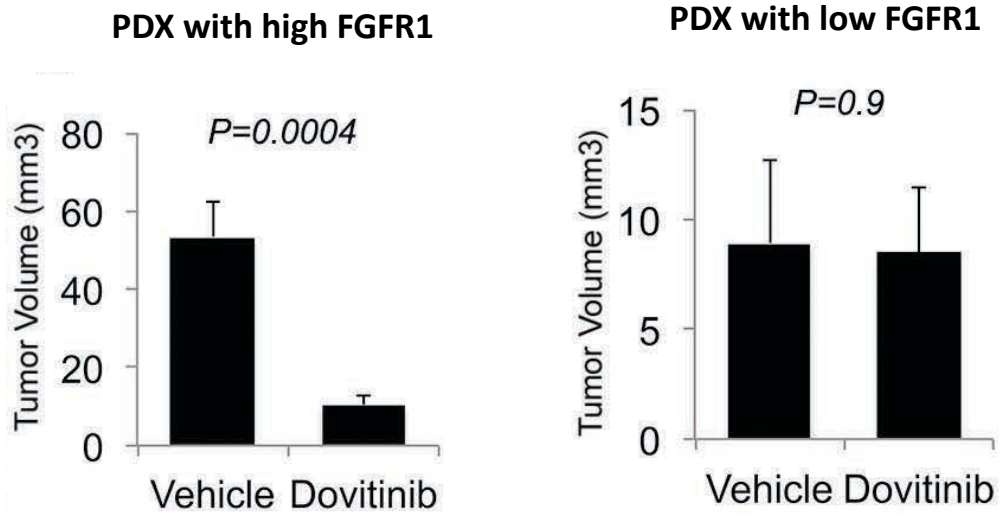
Dovitinib (TKI258) ⊣ FGFR, VEGFR

✓ Clinical activity in a subset of patients

men with castration resistant prostate cancer and bone metastases



✓ Antitumor activity in PDXs with high FGFR1



PDX: patient-derived xenograft

Wan et al Sci Transl Med 2014

Conclusion

FGF axis blockade is a new therapeutic target for men with castrate resistant PCa and bone metastases

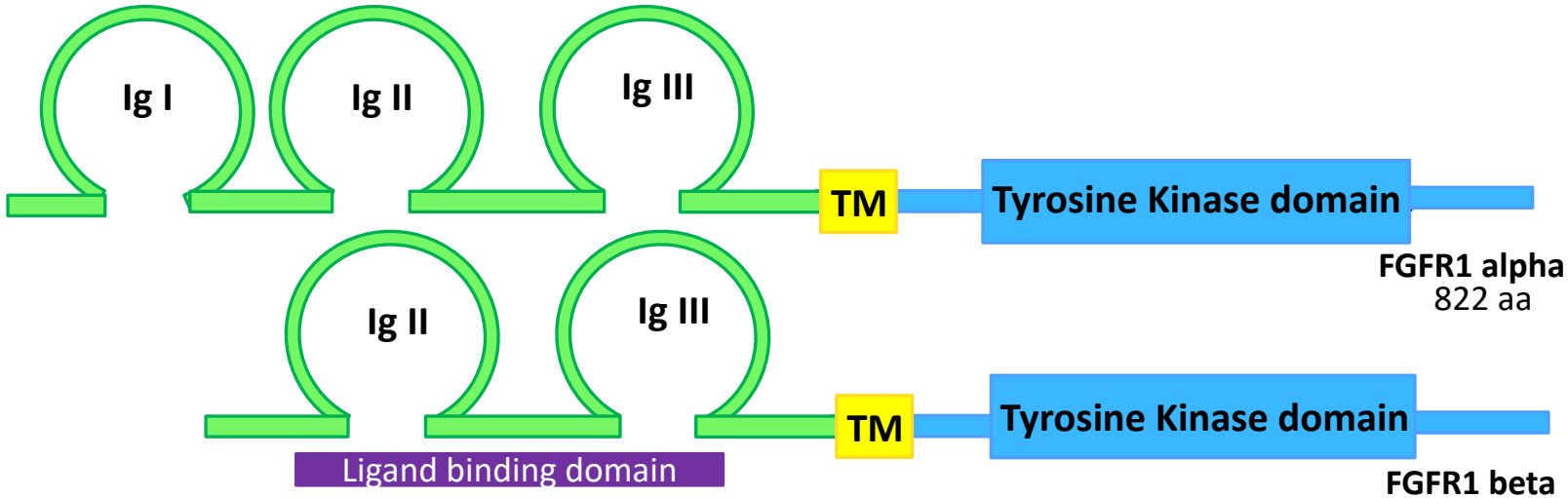
FGFR1 isoforms

Preliminary data

Different human PCa tissue samples express different FGFR1 isoforms

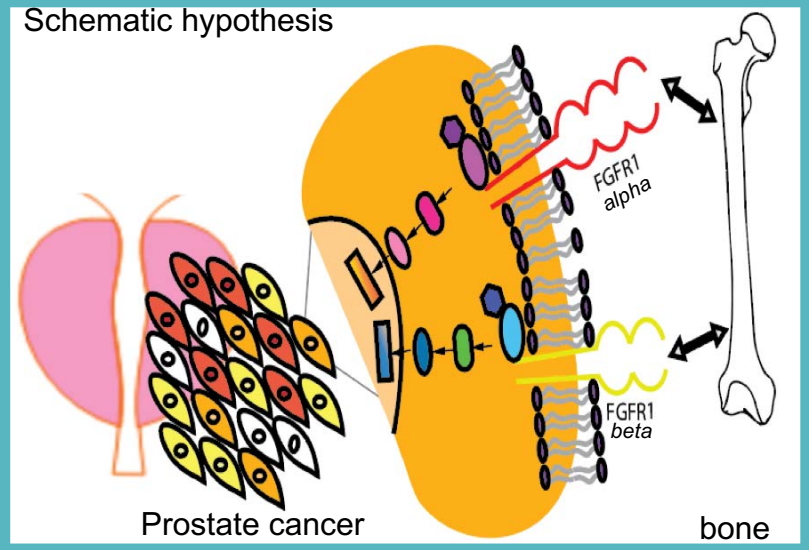
In collaboration with Dr. Chinnayian (UMHS)

FGFR1 isoforms



Johnson and Williams, 1993 Adv Cancer Res

FGFR1 isoforms have been associated with pancreatic cancer, breast cancer and glioblastoma (Bruno et al Hum Mol Genet 2004)



Hypothesis

- *FGFR1 alpha and beta confer different phenotypes to PCa cells, and this may partly explain PCa heterogeneity, pattern of progression, and differences in response to FGFR targeting*
- *FGFR1 mediates PCa cell–bone cell cross talk*

Goal

Investigate the molecular and clinical implications of the expression of FGFR1/FGFR1 isoforms in the pathogenesis of PCa bone metastases

Significance and Innovation

We propose that FGFR1 isoforms activate different genes or pathways in PCa

FGFR1- isoforms associated signature

Address clinical challenge

Identification of PCa patient candidates for FGFR blockade therapy

Specific Aims

Specific Aim 1. Analyze FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

Specific Aim 2. Assess the role of FGFR1 (and its isoforms) in the growth of PCa in bone, response to FGFR blockade and PCa-bone interaction

Approach

Specific Aim 1. Analyze FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

We will test our postulate that PCa tumors are heterogeneous in their expression of alpha and beta isoform levels throughout disease progression. Furthermore, we hypothesize that these two isoforms trigger activation of different associated gene signatures which cause, at least in part, this heterogeneity

Specific Aim 1. Analyze FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

(**a**) Mine the TCGA PCa datasets for FGFR1 isoforms

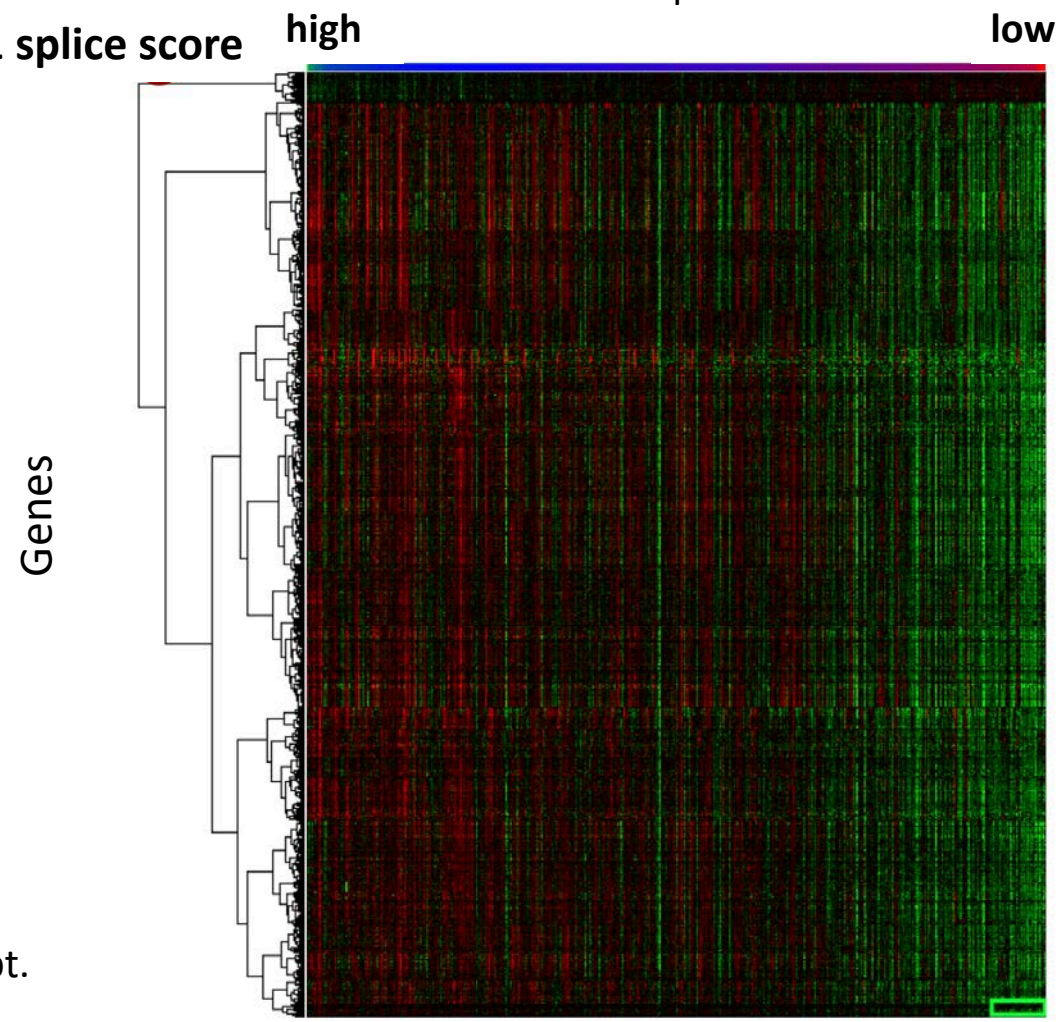
(**b**) Assess the expression of FGFR1 alpha and beta in clinical samples reflecting the progression of the disease (i.e. primary and metastatic PCa). For this last sub-aim, we will develop specific antibodies for each isoform

(**c**) Study the signaling cascade induced by FGFR1 alpha and beta by genetically manipulating FGFR1 isoform expression in PCa cells, and subsequently performing immunoblotting and reverse phase protein array (RPPA)

FGFR1 alpha and beta are associated with expression of different genes

Preliminary data

Alpha/beta ratio → **FGR1 splice score**



- correlated genes
+ correlated genes

In collaboration with Dr. Broom (Dept. of Bioinformatics and Comp Biology)

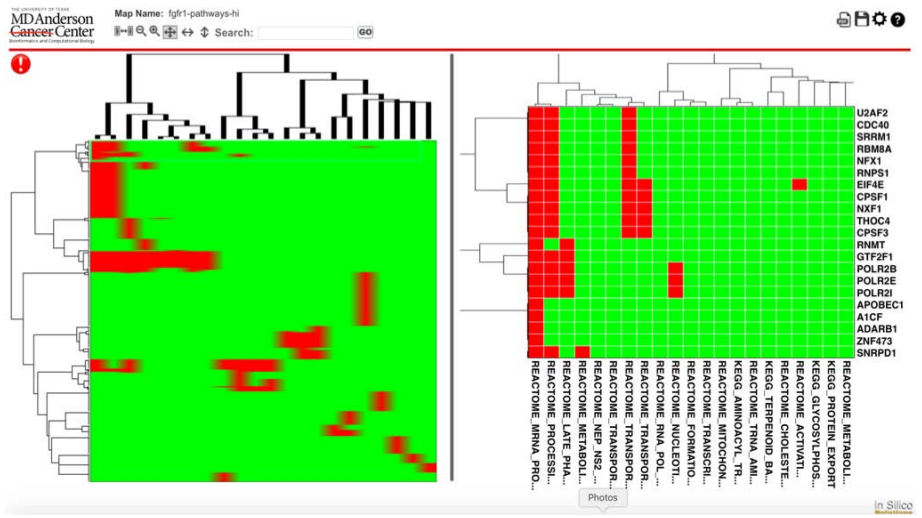
(a) To mine the TCGA PCa datasets to evaluate molecular and clinical correlates of FGFR1 isoforms

THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

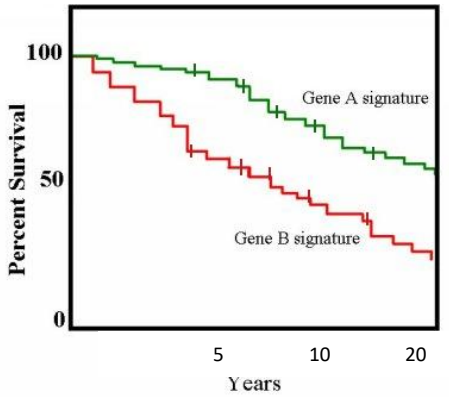
FGFR1 splice score

alpha → beta

Associated genes/pathways → Cluster heatmaps

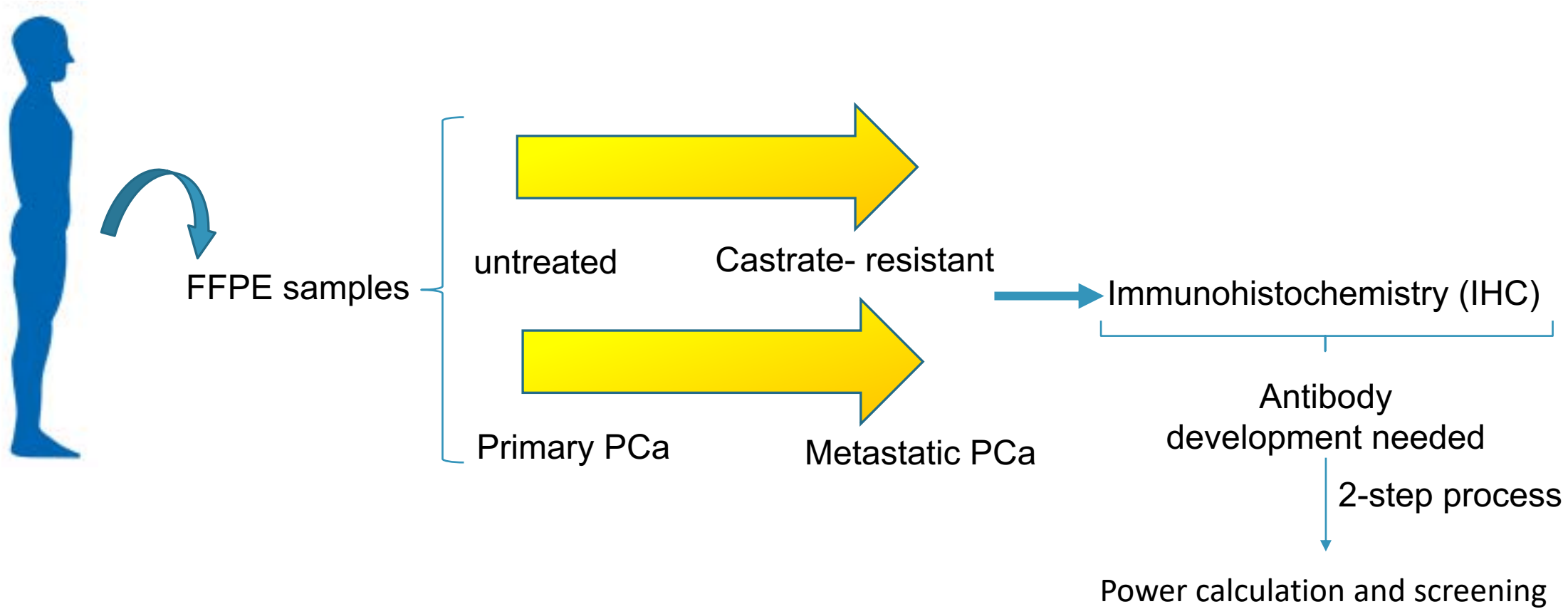


- clinical recurrence vs non-recurrence
- overall survival
- biochemical relapse-free survival
- time to progression after hormone treatment



In collaboration with Dr. Broom (Dept. of Bioinformatics and Comp Biology)

(b) To assess the expression of FGFR1 alpha and beta in clinical samples reflecting the progression of the disease

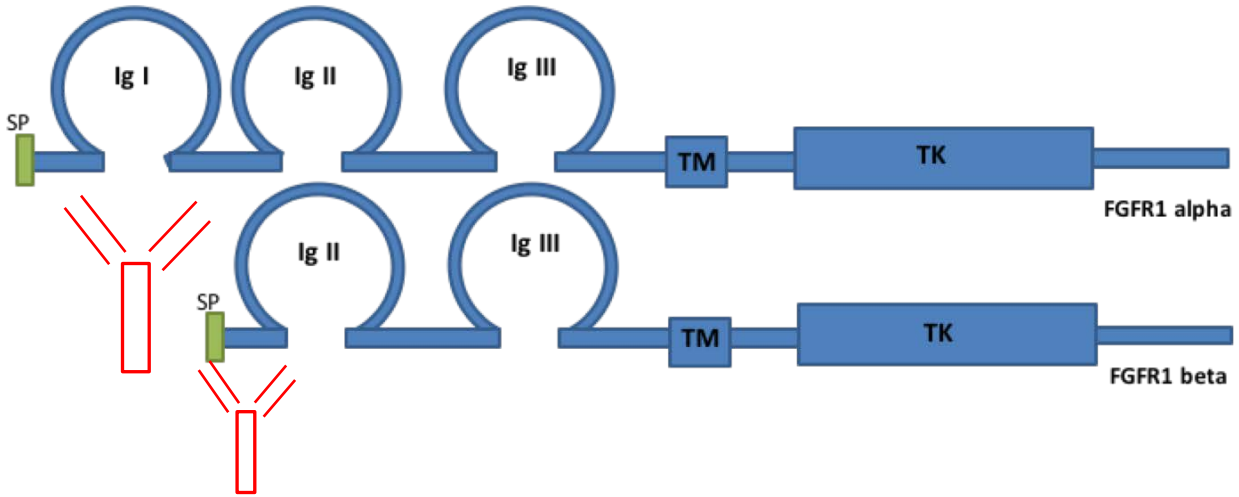


(b) To assess the expression of FGFR1 alpha and beta in clinical samples reflecting the progression of the disease

1. Develop Mouse Monoclonal Antibodies Using Hybridoma Technology

Creative Biolabs

Select antigens → BLAST & 3D modelling

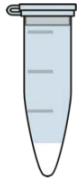


SP: Signal peptide

(b) To assess the expression of FGFR1 alpha and beta in clinical samples reflecting the progression of the disease

2. Test antibody specificity and sensitivity

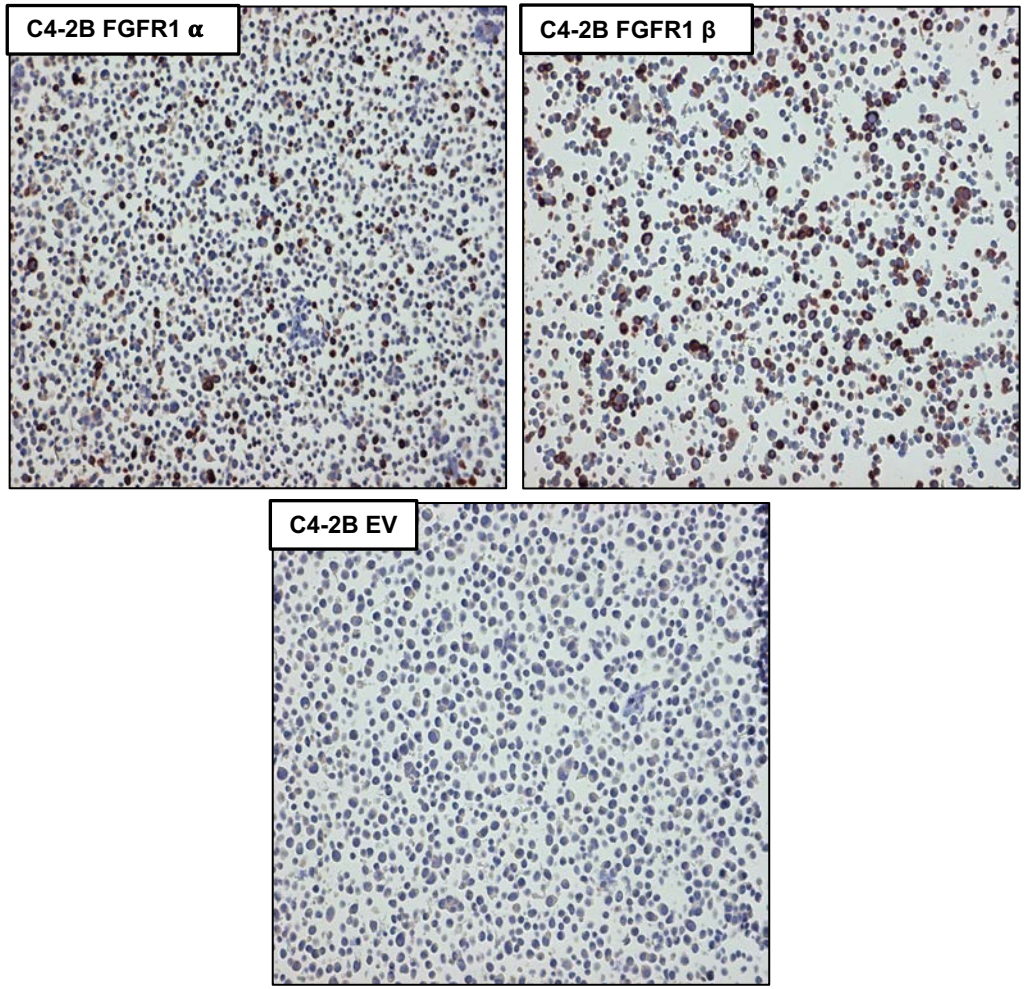
C4-2B expressing FGFR1 alpha, beta or EV



fix

Paraffin embed

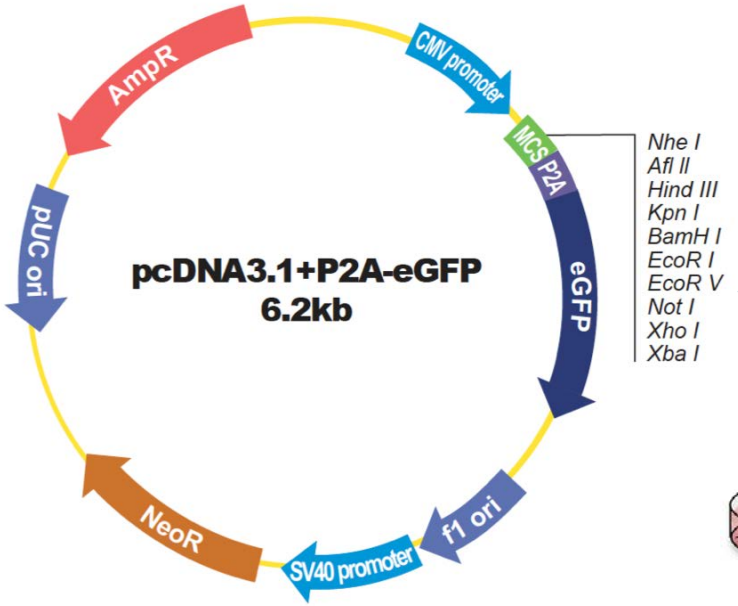
"IHC"



Total FGFR1 expression

(c) To study the signaling cascade induced by FGFR1 alpha and beta in PCa cells

1. Develop PCa cell lines expressing FGFR1 isoforms



Bicistronic vector

C4-2B & PC3
PCa cell lines

G418
selection
&
Cell sorting
(GFP+ cells)
x2

C4-2B EV ✓

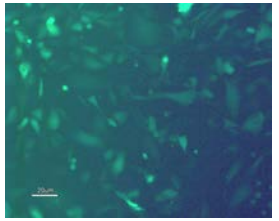
C4-2B FGFR1 alpha ✓

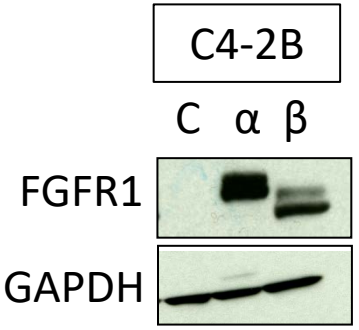
C4-2B FGFR1 beta ✓

PC3 EV ✓

PC3 FGFR1 alpha ✓

PC3 FGFR1 beta





(c) To study the signaling cascade induced by FGFR1 alpha and beta in PCa cells

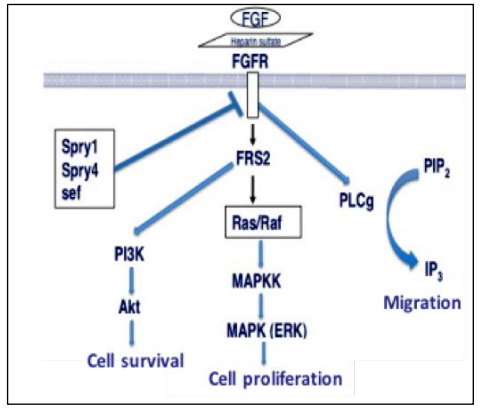
2. Induce signaling with FGF ligands

C4-2B EV
C4-2B FGFR1 alpha
C4-2B FGFR1 beta

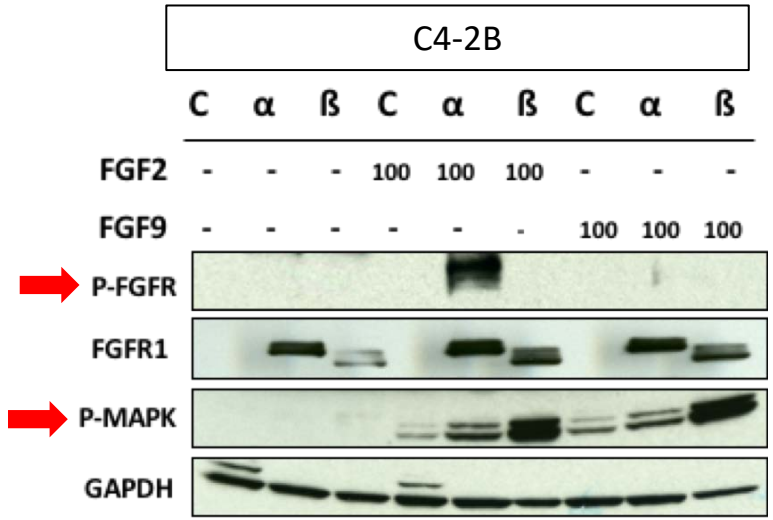
Serum-starvation → + HSPG → + FGF2/ FGF9

Targeted approach → Western blot

Discovery approach → RPPA → New targets



Known targets

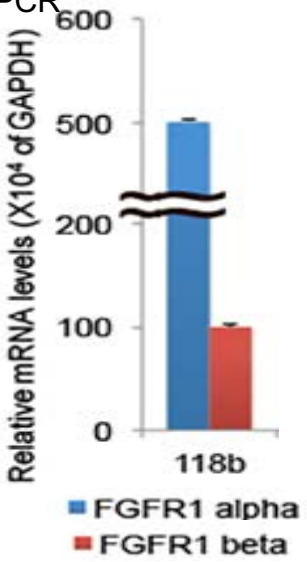


Same studies will be performed with PC3 sublines and with MDA PCa 118b patient-derived xenograft

(c) To study the signaling cascade induced by FGFR1 alpha and beta in PCa cells

3. Complementary approach

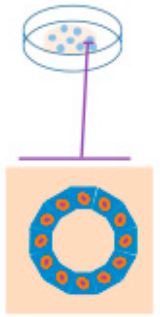
RT-qPCR



shRNA FGFR1/ scramble shRNA

MDA PCa 118b PDX

Culture in organoids



Gao et al Cell 2014

Western blot of FGFR1 targets

PDX: patient-derived xenograft

Expected results

Specific Aim 1. Analyze FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

(a) Find expression of different signaling pathways and genes linked to each FGFR1 isoform and information on clinical features associated

(b) Elucidate whether there is enrichment of a particular isoform (alpha or beta) during PCa progression

(c) Identify an FGFR1 isoform associated signature, resulting from different molecular outcomes of PCa cells expressing FGFR1 alpha or beta. Identify genes regulated by FGFR1 alpha but not beta and vice versa

Potential pitfalls and alternative approaches

a. Samples in TCGA may not have sufficient follow-up information or not enough cases with prevalent expression of each isoform to perform statistical analysis of progression

↓

Complement by mining other databases

b. Antibodies may lack specificity for IHC assay

↓

RNA in situ hybridization (ISH) in FFPE archived samples (collaboration- Dr. Palanisamy (HFHS))
3 probes: alpha-specific exon probe
skipping of the alpha-exon probe
a common probe for both FGFR1 alpha and beta

dual color assay → ratio

Another alternative → FGFR1 isoform expression profiling by ESI/MS (detection of specific peptides)

Specific Aim 2. Assess the role of FGFR1 (and its isoforms) in the growth of PCa in bone, response to FGFR blockade and PCa-bone interaction

We propose that FGFR1 accelerates the bone metastatic phenotype of PCa cells, which is orchestrated by the contribution of both isoforms

Specific Aim 2. Assess the role of FGFR1 (and its isoforms) in the growth of PCa in bone, response to FGFR blockade and PCa-bone interaction

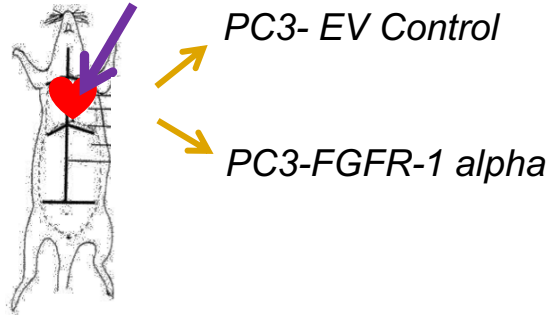
(**a**) Evaluate the metastatic dissemination of PCa cells after intracardiac injection of these cells in mice mediated by FGFR1 isoforms *in vivo*

(**b**) Assess the induction of PCa growth in bone by direct injection of PCa cells into the femur of mice and treated with a specific Pan-FGFR inhibitor, JNJ-42756493

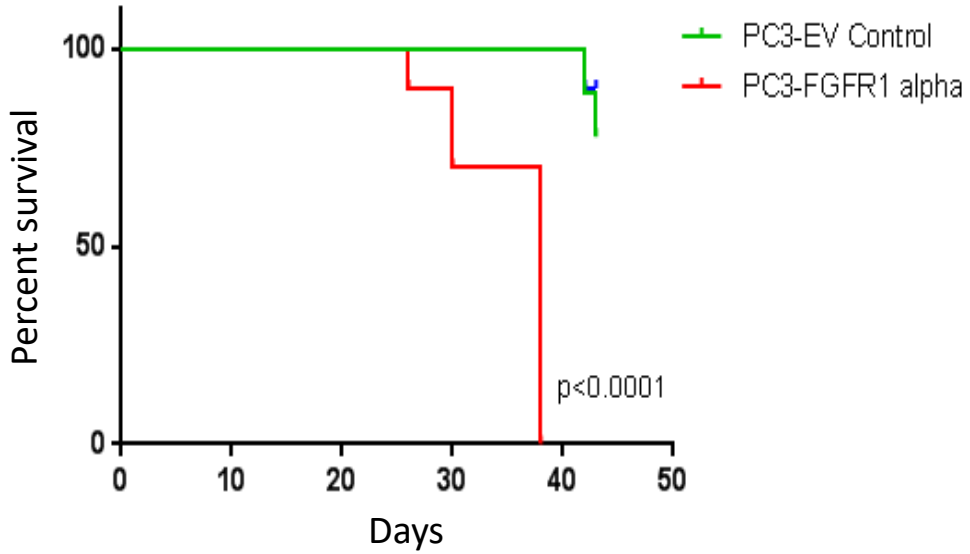
(**c**) Investigate the role of FGFR1 isoforms *in vitro* in the cross talk between PCa cells and bone cells (osteoblasts) by performing co-culture studies

Survival of mice was significantly reduced after intracardiac injection of PCa cells expressing FGFR1 alpha

Preliminary data

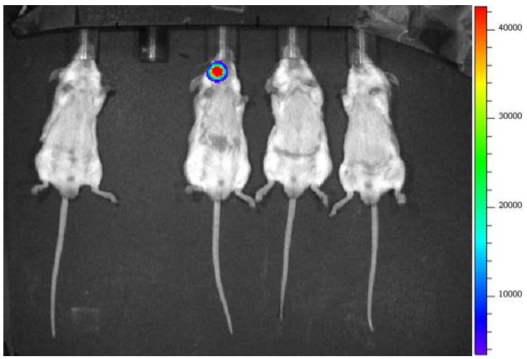


Survival curves

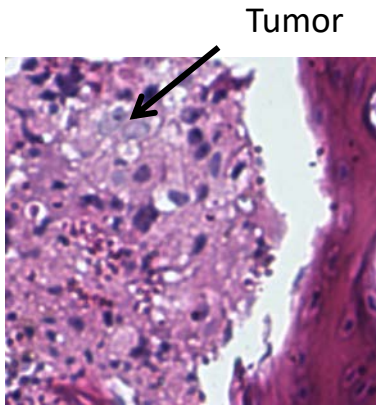


(a) To evaluate the metastatic dissemination of PCa cells mediated by FGFR1 isoforms *in vivo*

C4-2B mixed osteoblastic-osteolytic

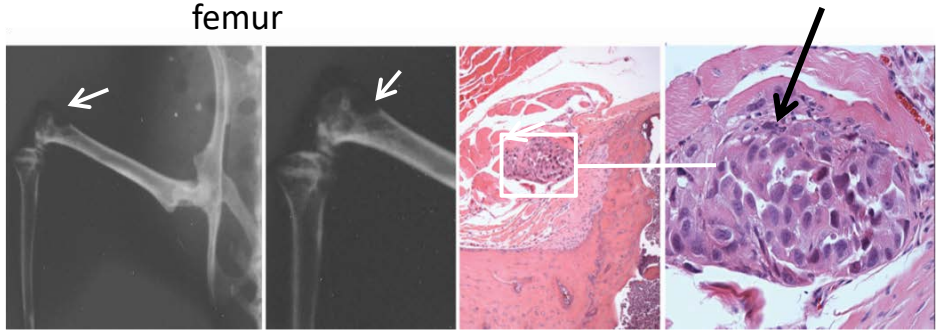


IVIS



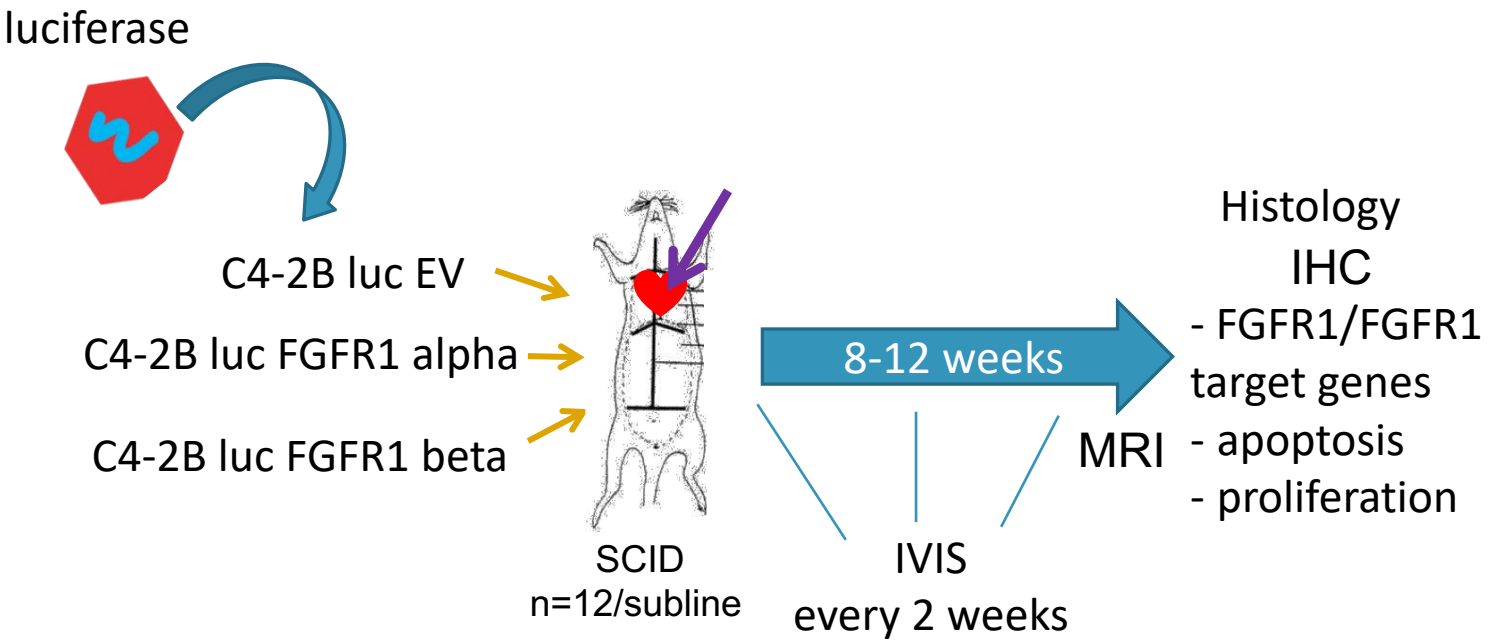
H&E

PC3: osteolytic



X-ray

H&E



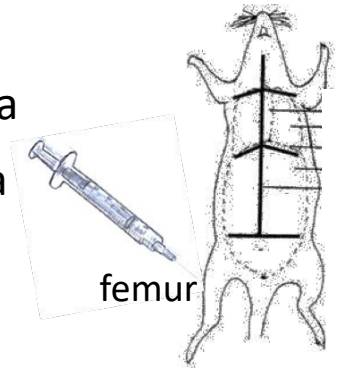
Similar studies will be performed with PC3 sublines (n=12/subline)

(b) To evaluate the induction of PCa growth in bone mediated by FGFR1 isoforms and the response of FGFR1 isoforms to treatment with a specific Pan-FGFR inhibitor

C4-2B EV

C4-2B FGFR1 alpha

C4-2B FGFR1 beta



SCID
n=6/subline

5 weeks

X-ray
every weeks

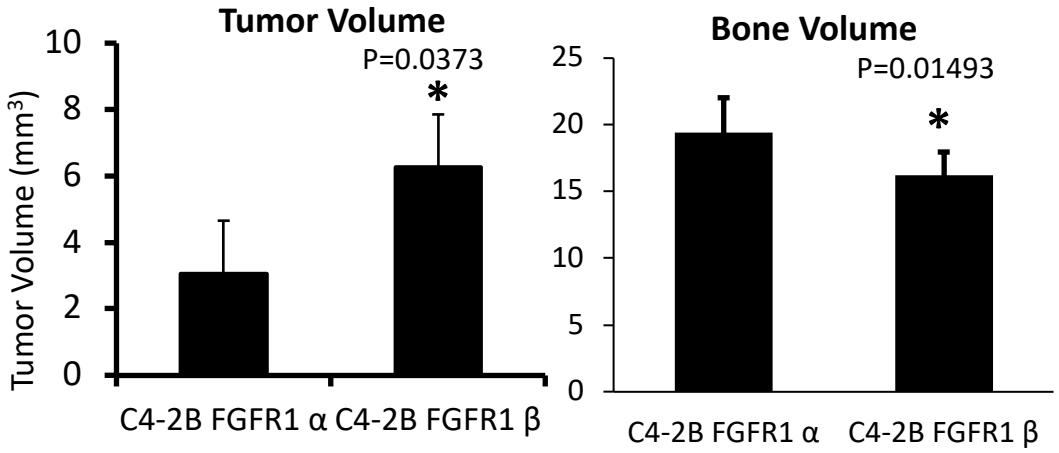
MRI

µCT

Bone histomorphometry

Histology

IHC - FGFR1 targets
- apoptosis
- proliferation



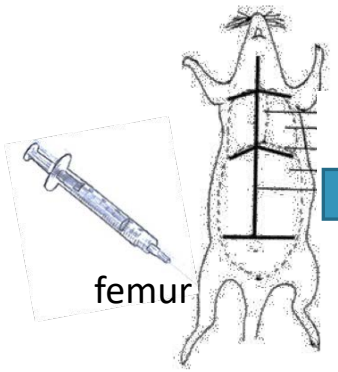
Same studies will be performed with PC3 sublines

(b) To evaluate the induction of PCa growth in bone mediated by FGFR1 isoforms and the response of FGFR1 isoforms to treatment with a specific Pan-FGFR inhibitor

C4-2B EV

C4-2B FGFR1 alpha

C4-2B FGFR1 beta



2 weeks

Vehicle (n=6)

Oral gavage administration TID

JNJ* (12.5 mg/kg body weight) (n=6)

3 weeks

X-ray every week

MRI

µCT

Bone histomorphometry

Histology

IHC

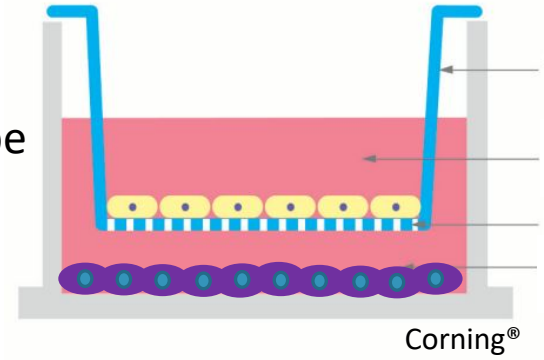
- FGFR1 targets
- apoptosis
- proliferation

Same studies will be performed with PC3 sublines

*JNJ-42756493 Pan-FGFR inhibitor (Janssen)

(c) To investigate the role of FGFR1 isoforms in the cross talk between PCa cells and bone cells

Boyden chamber-type system



C4-2B FGFR1 alpha, beta or EV

MC3T3
Mouse osteoblast precursor cell line

- Proliferation → [^{3}H]-thymidine & cell count
- Invasion → crystal violet stain & count (Matrigel/Collagen)
- Migration → crystal violet stain & count
- Signaling pathways → Western blot

Same studies will be performed with PC3 sublines

Expected results

Specific Aim 2. Assess the role of FGFR1 (and its isoforms) in the growth of PCa in bone, response to FGFR blockade and PCa-bone interaction

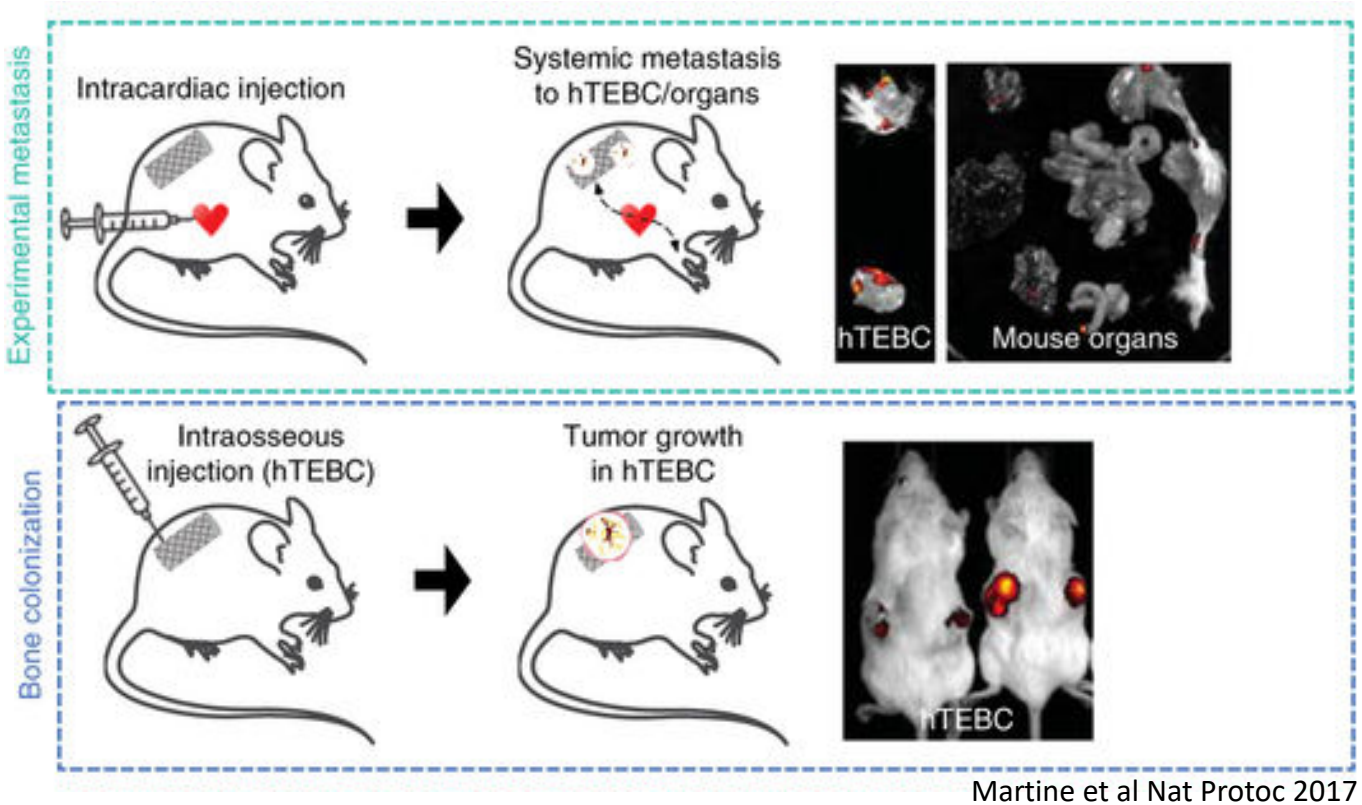
(a) Determine whether FGFR1 or a specific FGFR1 isoform mediates the metastatic progression of PCa cells; and find a direct correlation between FGFR1 expression and PCa cell aggressiveness

(b) FGFR1 isoforms induce different growth rates or bone reaction. Also a long-term goal of these studies is to identify factors that predict response to FGFR blockade in men with PCa

(c) Cells expressing the isoforms will be more favored by the interaction with the bone, hence resulting in an increased effect in the parameters assessed when compared to control. Also, isolate the individual contribution of each of the isoforms in the interaction with bone-forming cells

Potential pitfall and alternative approaches

To better mimic species-specific mechanisms: hTEBC model

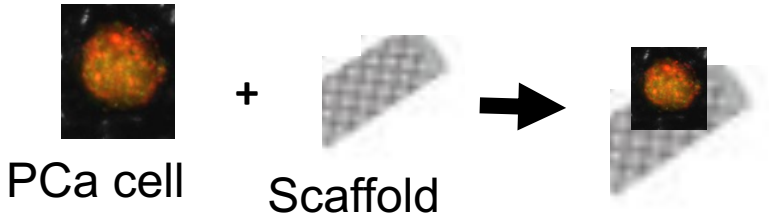


Martine et al Nat Protoc 2017

Analyze the metastatic dissemination of PCa cells mediated by FGFR1 isoforms (a)

Monitor PCa lesions and bone colonization (b) and therapy response (b)

Potential pitfall and alternative approaches



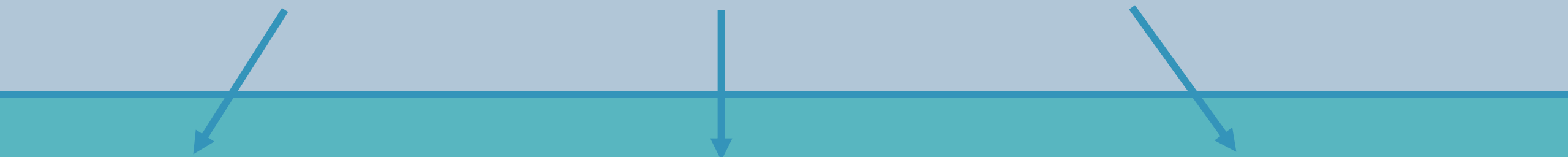
PCa cell spheroid + Scaffold + human osteoblasts → Tumor growth and invasion (c)

(c) Co-culture: effect on osteoblasts → markers of proliferation and differentiation of osteoblasts

- [^{3}H]-thymidine/ WTS-1
- ALP activity
- RUNX2, ALP, COL1A1, BMPs
- Osteocalcin ELISA
- Von kossa

Conclusive statement

A thorough analysis of the effects exerted by FGFR1 in PCa and the comprehension of the molecular mechanisms by which FGFR1 and its isoforms act, can contribute to more accurate therapeutic application of an established/developing treatment for this disease, in particular for the aggressive stage



Recognize FGFR1 blockade responders

Develop new therapies targeting FGFR

Identify predictive biomarkers of response to treatment

Thank you

Navone's Lab

Dr. Nora Navone

Jun Yang

Michael Starbuck

Peter Shepherd

Dr. Justin Roberts

Candidacy Exam Committee

Dr. Pierre McCrea (Chair)

Dr. Fen Wang

Dr. Anil Sood

Dr. Juan Fueyo

Dr. David Rowley

Advisory Committee

Dr. Nora Navone

Dr. Fen Wang

Dr. Pierre McCrea

Dr. Gary Gallick

Dr. Anil Sood

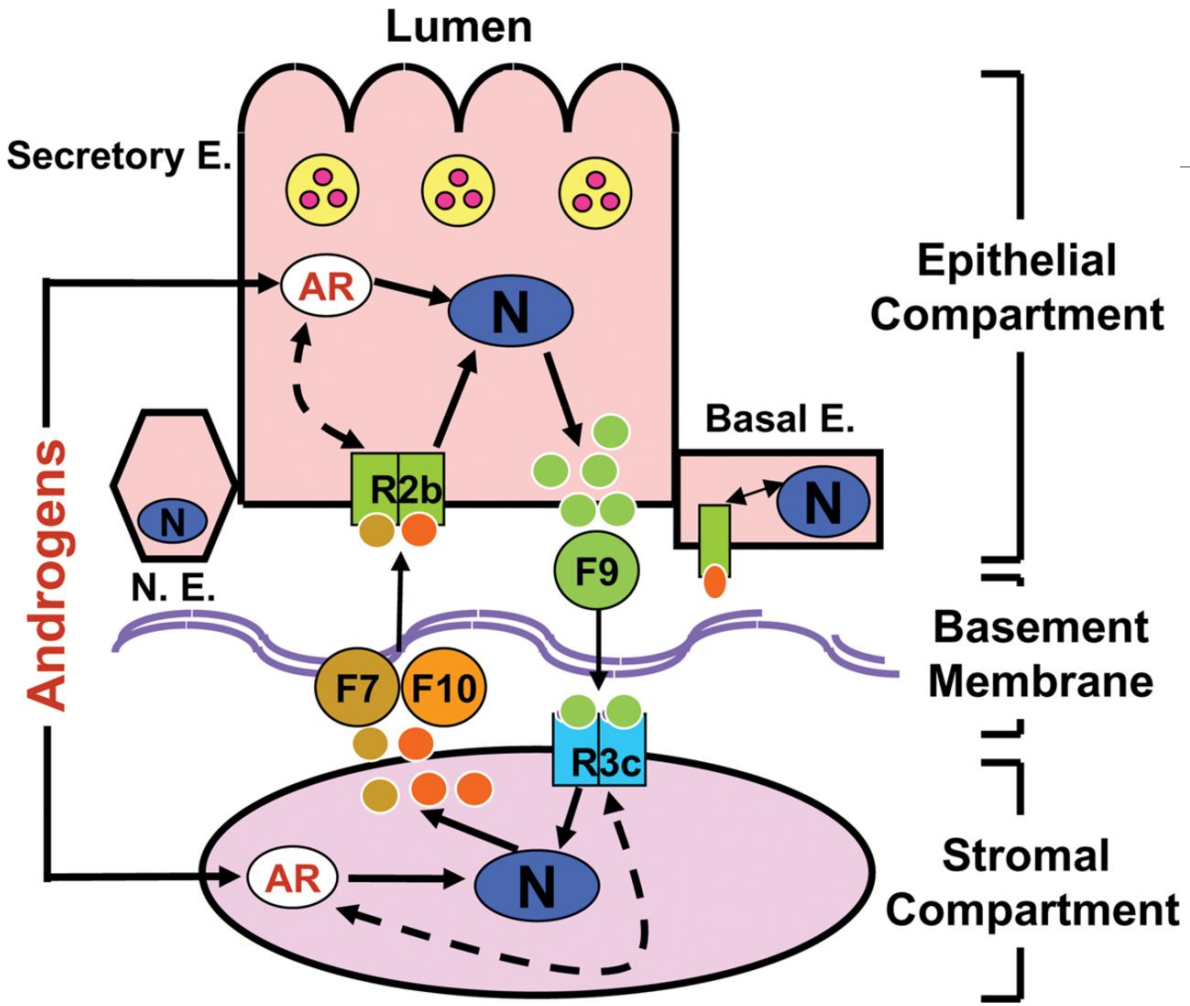
Additional

FGFR expression in normal prostate

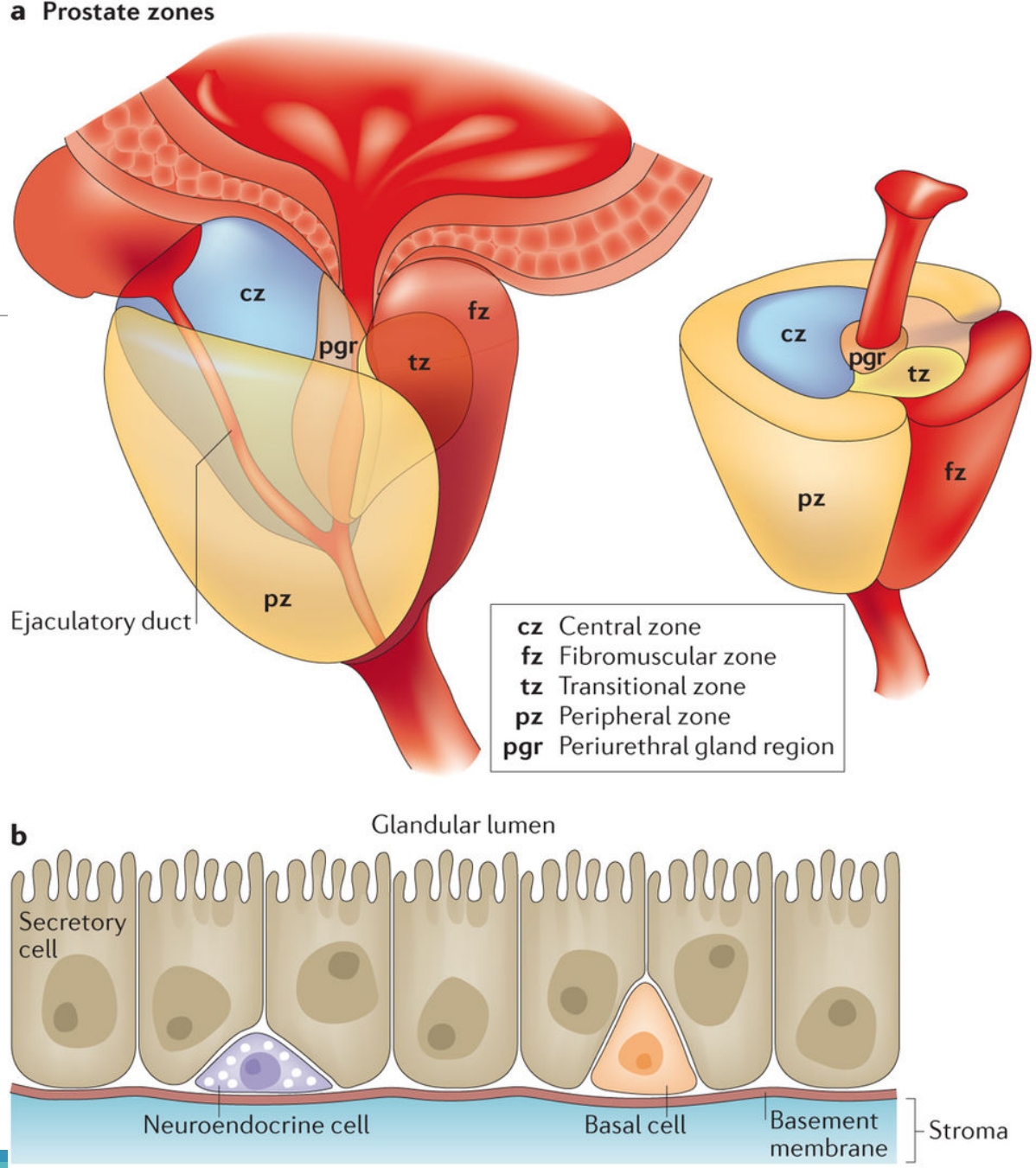
The prostate is composed of stromal cells and epithelial cells. Stromal cells secrete paracrine factors for the maintenance and growth of the epithelium, some of which are under the control of androgens. FGF2, 7 and 9 are the main FGFs that the stromal cells secrete. Prostate epithelial cells express multiple FGF receptors. **FGFR1 and FGFR2 are expressed in the basal epithelial cells of the prostate but not the luminal cells**. FGFR3 IIIb and FGFR4 are also expressed in normal epithelium. FGFR1 is present exclusively as the IIIc isoform, while FGFR2 is present exclusively as the IIIb (FGF7 specific) isoform in the epithelium. FGFR3 is also present in prostatic epithelium, predominantly in the IIIb isoform. FGFR4 is also expressed in prostatic epithelium in the luminal epithelial cells (review in Kwabi-Addo et al., 2004).

http://atlasgeneticsoncology.org/Genes/FGFR1ID113.html

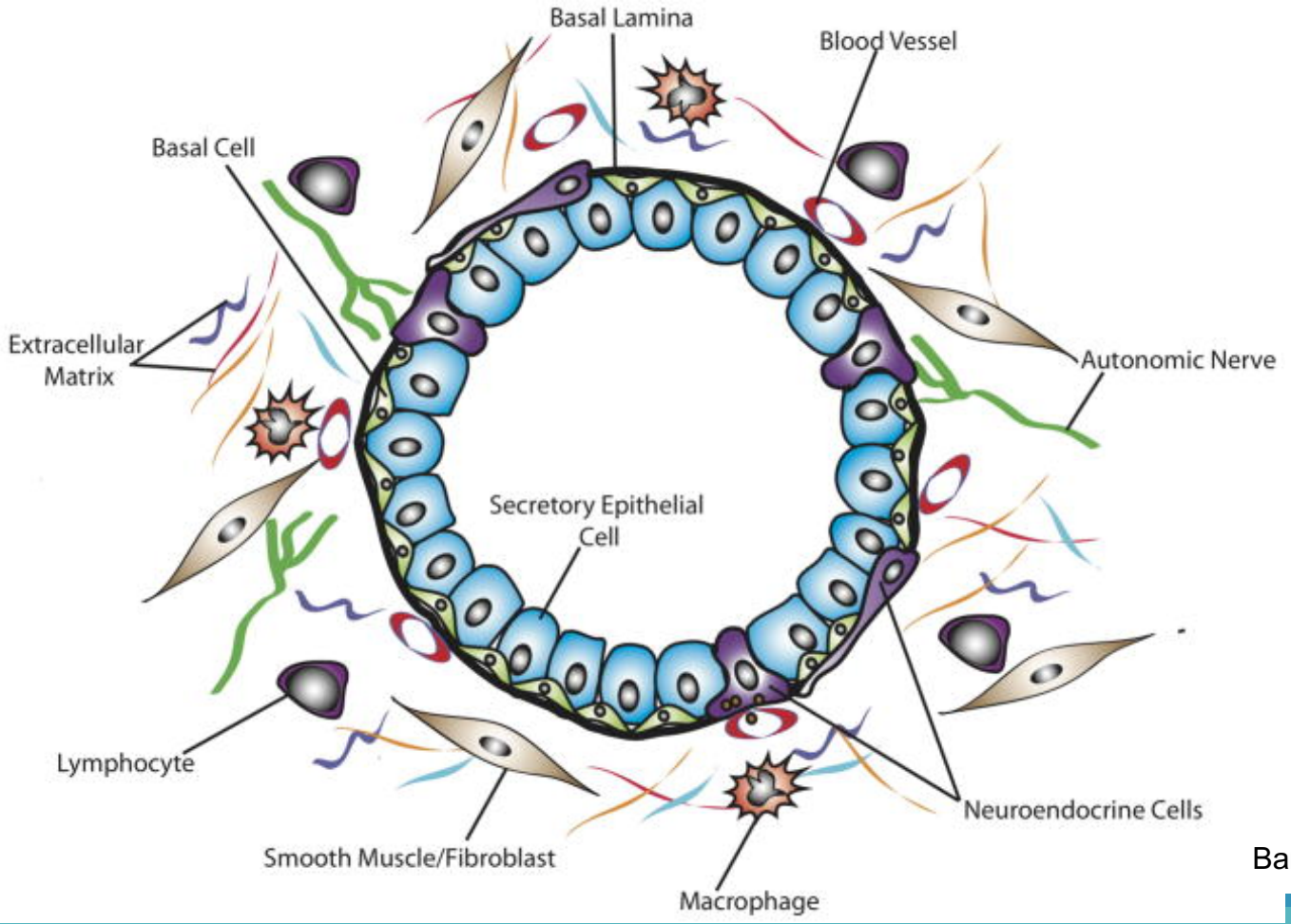
FGF in prostate development and epithelial- stromal interactions



Lin and Wang, Bioscience Reports 2010

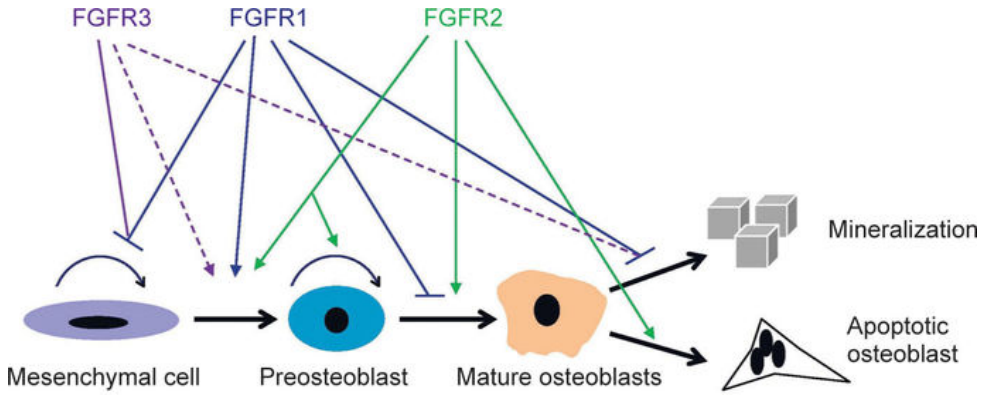

a Prostate zones
cz
fz
pgr
tz
pz
Ejaculatory duct
cz
pgr
tz
fz
pz
cz Central zone
fz Fibromuscular zone
tz Transitional zone
pz Peripheral zone
pgr Periurethral gland region
b
Glandular lumen
Secretory cell
Neuroendocrine cell
Basal cell
Basement membrane
Stroma

Cellular components of the human prostate gland

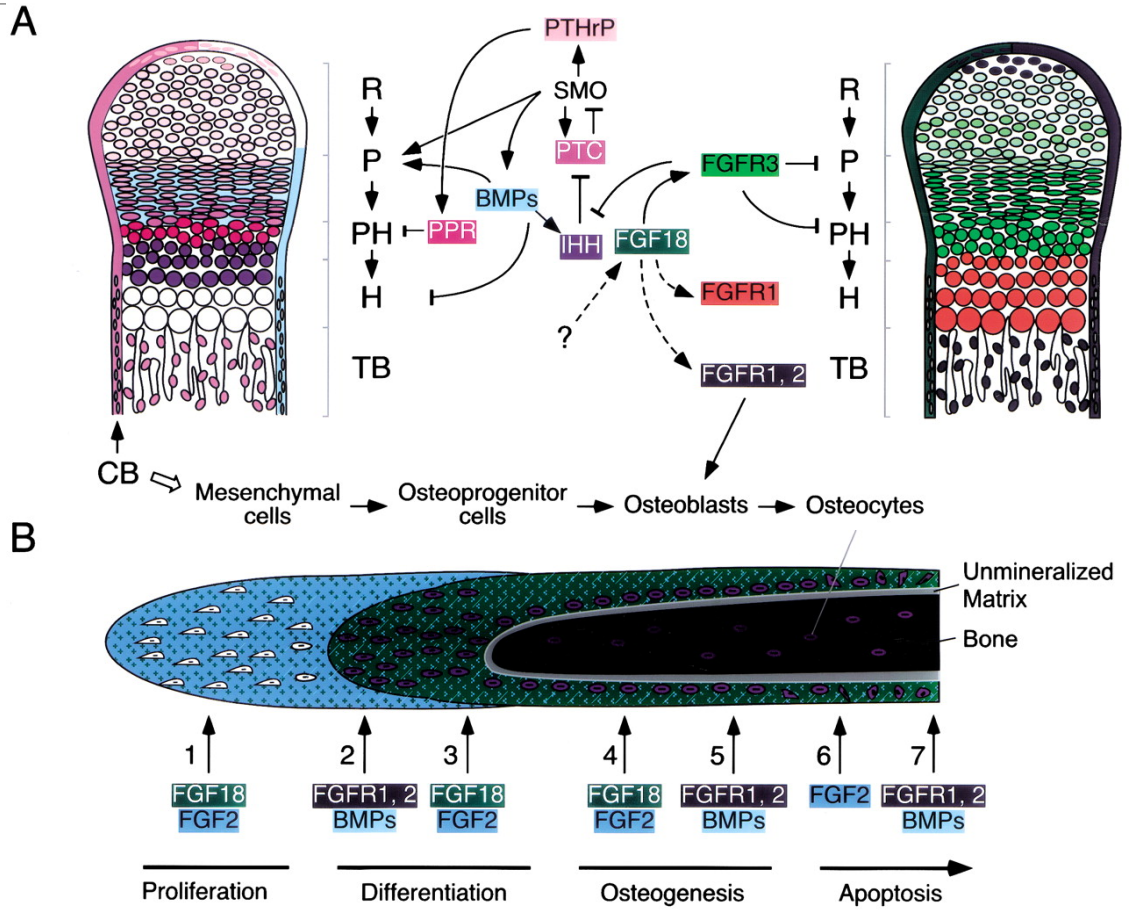


Barron and Rowley, Endocr Relat Cancer. 2012

FGF in bone development



Su et al Bone Reserach 2014



Ornitz Genes & Develop 2002

http://atlasgeneticsoncology.org/Genes/FGFR1ID113.html

FGFR in PCa

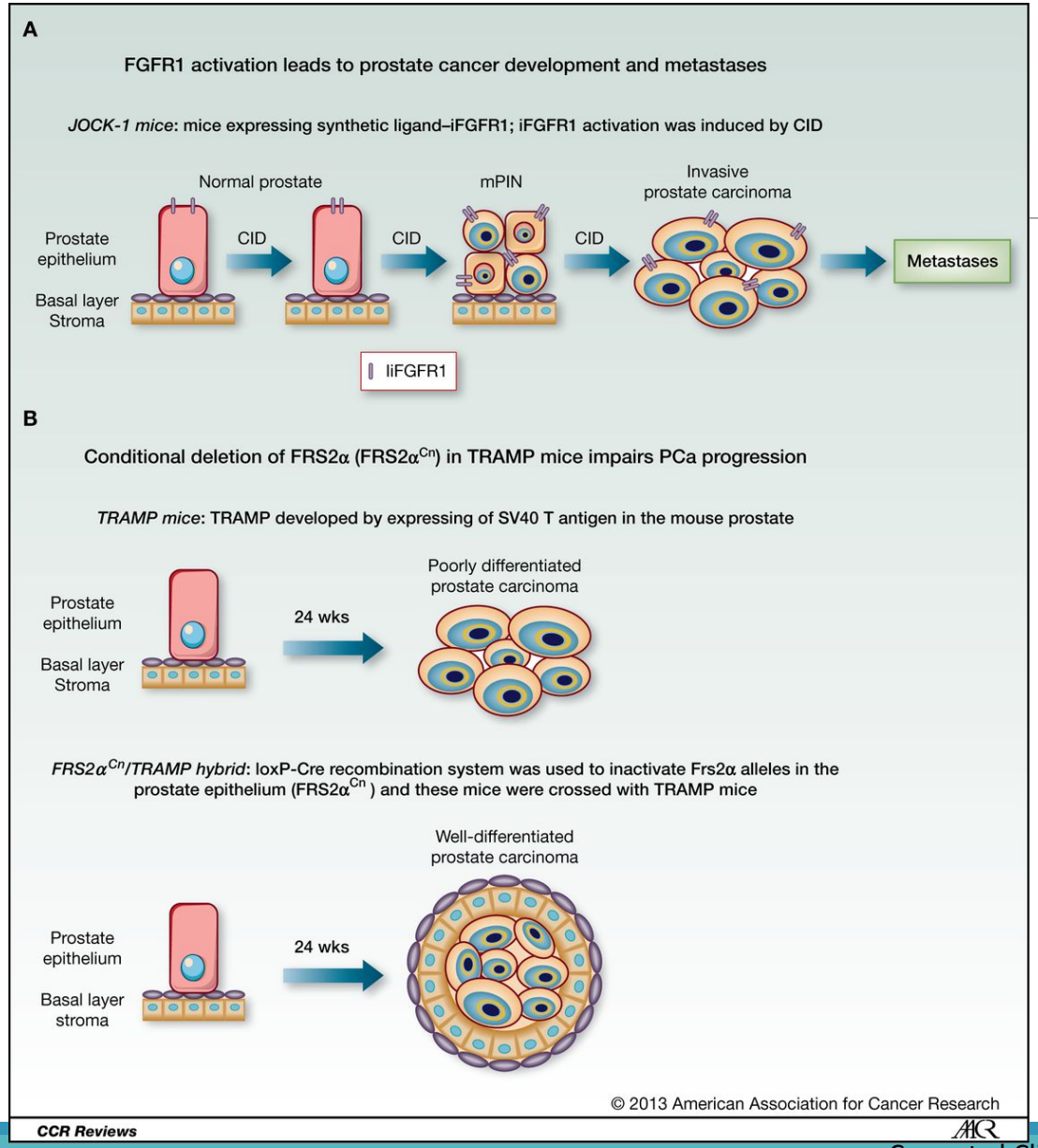
FGFR1 is over-expressed in benign prostatic hyperplasia whereas FGFR2-IIIc and FGFR3 are not (Boget et al., 2001). Transcripts for FGFR1 are found in prostate cancer cells (Shain et al., 2004), while FGFR2 is down regulated (Naimi et al., 2002). Both FGFR1 and FGFR4 have been found overexpressed in a study of 138 malignant prostates (Sahadevan et al., 2007). Chronic activation of FGFR1 in mouse prostate epithelial cells induces progressive prostate intraepithelial neoplasia (Wang et al., 2004). The FGFR1-IIIb isoform was expressed in all cases of prostate cancer, while FGFR1-IIIc mRNA was not. FGFR1-IIIb transcripts were detected in four out of six cases of benign prostatic hyperplasia (Leung et al., 1997). Although FGFR1 was found overexpressed in prostate cancer, it was without any significant correlation to clinical parameters including tumour grade, stage, and outcome, according to some studies (Leung et al., 1997; Giri et al., 1999). Conversely, Devilard et al., 2006 found that FGFR1, TACC1 (8p11; transforming, acidic coiled-coil containing protein 1) and WT1 (11p13; Wilms tumor 1) were expressed at higher level in prostate carcinoma samples than in benign prostate tissue, at both mRNA and protein levels, especially so in pT3 and N1/M1 samples. Transfection and expression of FGFR1 in premalignant cells accelerated progression to the malignant phenotype; restauration of FGFRIIIb in cells expressing FGFR1 restored epithelial cell differenciation (Feng et al., 1997). FGF2 was found in cells surrounding the cancer cells (fibroblasts and endothelium), and FGFR1 and FGFR2 expression were found increased in poorly differenciated prostate cancers, which would enhence the response of cancer cells to FGF2 (Giri et al., 1999).

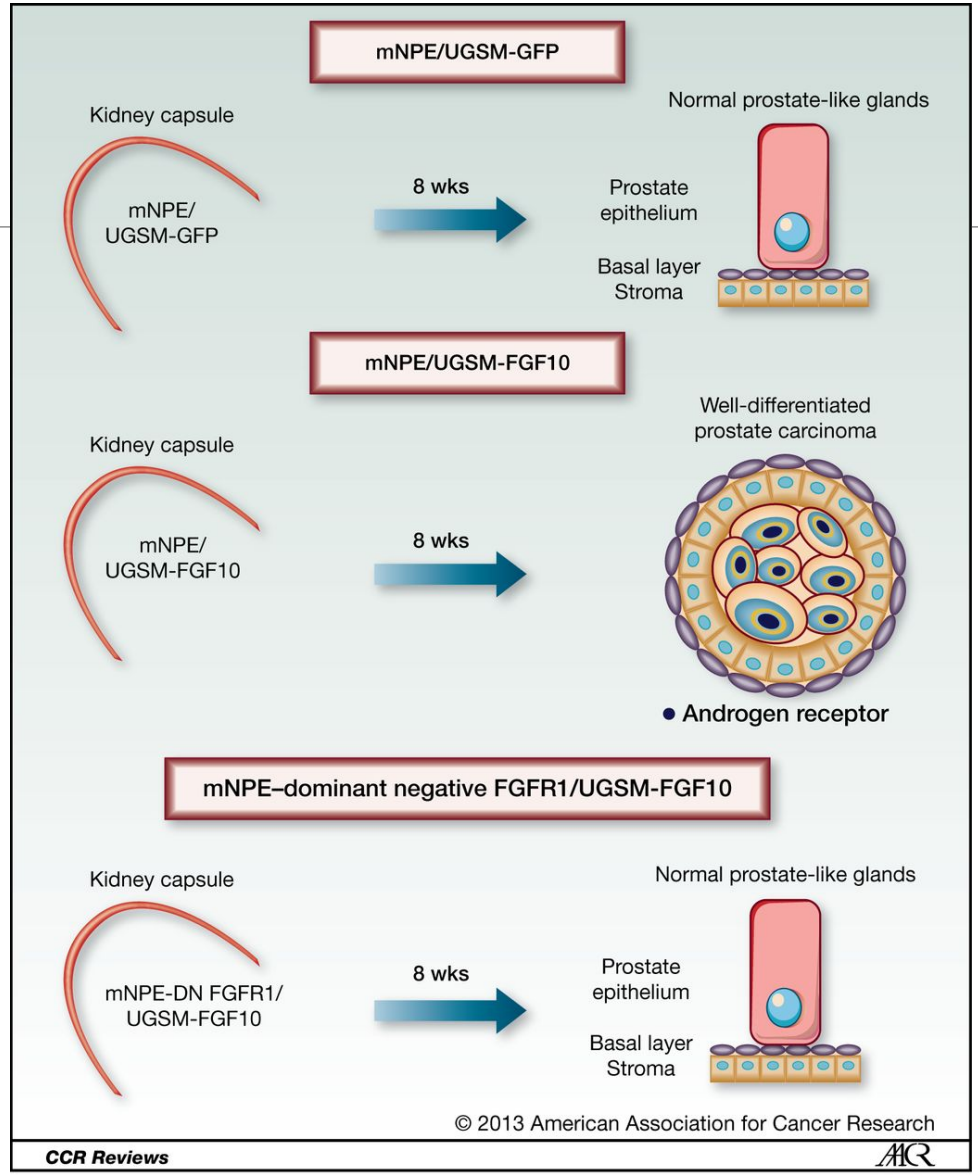
http://atlasgeneticsoncology.org/Genes/FGFR1ID113.html

FGFR in PCa

Activation of inducible FGFR1 led to epithelial-to-mesenchymal transition (like with breast cells) and progression to adenocarcinoma in the mouse. Mice not only developed well-differentiated adenocarcinoma, but also exhibited several distinct malignant phenotypes: prostatic intraepithelial neoplasia, adenocarcinoma, transitional sarcomatoid-carcinoma, and frank sarcoma. Mice developed a greater incidence of a transitional sarcomatoid carcinoma with increasing age, consistent with the appearance of an epithelial-mesenchymal transition. Experimental up-regulation of FGFR1 provoked SOX9 increase. SOX9 (17q23; SRY (sex determining region Y)-box 9) is known to act with SNAI1 (20q13; snail homolog 1 (Drosophila)) and SNAI2 (8q12; snail homolog 2 (Drosophila)) to reduce CDH1, leading to a loss of cell-cell contact and increased migration (Acevedo et al., 2007). Enhanced mesenchymal expression of FGF10 leeds to the formation of cancers from murine prostate cells. Inhibition of FGFR1 signaling by dominant-negative FGFR1 reverts FGF10-induced adenocarcinoma (Memarzadeh et al., 2007). Amplification of FGFR1 and many other loci were found associated with the development of hormone resistance of the cancer cells (Edwards et al., 2003). SPRY1 (4q28; Sprouty1) and SPRY2 (13q31; Sprouty2) mRNAs, antagonists of FGF signaling (see above), are decreased in human prostate cancer (Kwabi-Addo, Wang et al., 2004; Fritzsche et al., 2006). Inducible FGFR1 provokes angiogenesis in the prostate of mice; ANGPT1 and ANGPT2(angiopoietins 1 and 2, 8q23 and 8p23 respectively) were regulated by FGFR1 signaling and differentially expressed (Winter et al., 2007).

FGF in PCa





Corn et al Clin Can Res 2013

FGFR1 isoforms alpha and beta

Glioblastoma

- FGFR1 alpha poorly expressed in normal glia. FGFR1 beta preferentially expressed in malignant astrocytomas (n=22)
- FGFR1beta: 10-fold higher affinity for FGF1 and FGF2 than FGFR1 alpha
- Targeted inclusion of alpha-exon to glioblastoma: no discernable effect on cell growth in culture, but associated with increase in unstimulated caspase 3 & 7activity (Bruno et al, 2004)
- SFPQ (splicing factor proline/glutamine-rich, alias PTBP, polypyrimidine tract-binding protein):regulator of FGFR1 splicing. SFPQ expression was found strongly increased in malignant glioblastoma multiforme tumors, but not in a low-grade astrocytoma case (Jin et al, 2000)

Breast cancer

- FGFR1 beta preponderant in breast cancer, and FGFR1 alpha in normal breast cells (Luqmani et al., 1995)

Pancreatic cancer

- FGFR1alpha expressed in normal pancreatic tissue. Pancreatic adenocarcinomas overexpress FGFR1 beta in ≈90% cases
- Overexpression of FGFR1 alpha inhibits pancreatic adenocarcinoma cells (Vickers et al., 2002)

http://atlasgeneticsoncology.org/Genes/FGFR1ID113.html

Expression of 2 variant forms of fibroblast growth factor receptor 1 in human breast

The expression of variant mRNAs encoding isoforms of fibroblast growth factor receptor (FGFR) 1 with either 2 or 3 Ig-like loops in the extracellular domain was investigated in human breast tissues and cell lines using a polymerase chain reaction amplification method. Almost all tissues contained both forms of FGFR1, but cancers (n = 137) had a significantly lower proportion of the transcript that encoded the full 3-loop form compared with non-malignant biopsies (n = 34). This was confirmed using microdissected populations of normal and cancerous cells from frozen tissue sections. A high ratio of the 2-to 3-loop form was found to be predictive of reduced relapsefree survival. In both groups, however, the predominant form of FGFR1 was that encoding the 2-loop receptor. Cell lines derived from a variety of tissues, including breast, also co-expressed both variants of FGFR1, suggesting their presence within the same cell type. Again, there was a similar preponderance of the shorter isoform. Our results were confirmed at the protein level, where out of 5 cancers analysed 4 expressed more of the 2-loop form than the 3-loop form. Our findings suggest that cells may normally simultaneously express several splice variants of FGFR1, and aberrant expression or a change in their relative amounts (i.e., in malignancy) could contribute to modified responses to either autocrine or paracrine factors.

Luqmani et al Int Journ Can 1995

Fibroblast growth factor receptor splice variants are stable markers of oncogenic transforming growth factor β1 signaling in metastatic breast cancers

INTRODUCTION: EMT and MET facilitate breast cancer (BC) metastasis; however, stable molecular changes that result as a consequence of these processes remain poorly defined. Therefore, with the hope of targeting unique aspects of metastatic tumor outgrowth, we sought to identify molecular markers that could identify tumor cells that had completed the EMT:MET cycle.
METHODS: An in vivo reporter system for epithelial cadherin (E-cad) expression was used to quantify its regulation in metastatic BC cells during primary and metastatic tumor growth. Exogenous addition of transforming growth factor β1 (TGF-β1) was used to induce EMT in an in situ model of BC. Microarray analysis was employed to examine gene expression changes in cells chronically treated with and withdrawn from TGF-β1, thus completing one full EMT:MET cycle. Changes in fibroblast growth factor receptor type 1 (FGFR1) isoform expression were validated using PCR analyses of patient-derived tumor tissues versus matched normal tissues. FGFR1 gene expression was manipulated using short hairpin RNA depletion and cDNA rescue. Preclinical pharmacological inhibition of FGFR kinase was employed using the orally available compound BGJ-398.
RESULTS: Metastatic BC cells undergo spontaneous downregulation of E-cad during primary tumor growth, and its expression subsequently returns following initiation of metastatic outgrowth. Exogenous exposure to TGF-β1 was sufficient to drive the metastasis of an otherwise in situ model of BC and was similarly associated with a depletion and return of E-cad expression during metastatic progression. BC cells treated and withdrawn from TGF-β stably upregulate a truncated FGFR1-β splice variant that lacks the outermost extracellular immunoglobulin domain. Identification of this FGFR1 splice variant was verified in metastatic human BC cell lines and patient-derived tumor samples. Expression of FGFR1-β was also dominant in a model of metastatic outgrowth where depletion of FGFR1 and pharmacologic inhibition of FGFR kinase activity both inhibited pulmonary tumor outgrowth. Highlighting the dichotomous nature of FGFR splice variants and recombinant expression of full-length FGFR1-α also blocked pulmonary tumor outgrowth.
CONCLUSION: The results of our study strongly suggest that FGFR1-β is required for the pulmonary outgrowth of metastatic BC. Moreover, FGFR1 isoform expression can be used as a predictive biomarker for therapeutic application of its kinase inhibitors.

Wendt et al Breast Can Res 2014

Correction of aberrant *FGFR1* alternative RNA splicing through targeting of intronic regulatory elements

Alternative RNA splicing is now known to be pervasive throughout the genome and a target of human disease. We evaluated if targeting intronic splicing regulatory sequences with antisense oligonucleotides could be used to correct aberrant exon skipping. As a model, we targeted the intronic silencing sequence (ISS) elements flanking the alternatively spliced α-exon of the endogenous fibroblast growth factor receptor 1 (*FGFR1*) gene, which is aberrantly skipped in human glioblastoma. Antisense morpholino oligonucleotides targeting either upstream or downstream ISS elements increased α-exon inclusion from 10% up to 70% *in vivo*. The effect was dose dependent, sequence specific and reproducible in several human cell lines, but did not necessarily correlate with blocking of protein association *in vitro*. Simultaneous targeting of the ISS elements had no additive effect, suggesting that splicing regulation occurred through a shared mechanism. Broad applicability of this approach was demonstrated by similar targeting of the ISS elements of the human *hnRNPA1* gene. The correction of *FGFR1* gene splicing to >90% α-exon inclusion in glioblastoma cells had no discernable effect on cell growth in culture, but was associated with an increase in unstimulated caspase-3 and -7 activity. The ability to manipulate endogenously expressed mRNA variants allows exploration of their functional relevance under normal and diseased physiological states.

Bruno et al, Hum Mol Gen 2004

Differential expression of two fibroblast growth factor-receptor genes is associated with malignant progression in human astrocytomas

Malignant astrocytomas, which are highly invasive, vascular neoplasms, compose the majority of nervous system tumors in humans. Elevated expression of fibroblast growth factors (FGFs) in astrocytomas has implicated the FGF family of mitogens in the initiation and progression of astrocyte-derived tumors. In this study, we demonstrated that human astrocytomas undergo parallel changes in FGF-receptor (FGFR) expression during their progression from a benign to a malignant phenotype. FGFR type 2 (BEK) expression was abundant in normal white matter and in all low-grade astrocytomas but was not seen in malignant astrocytomas. Conversely, FGFR type 1 (FLG) expression was absent or barely detectable in normal white matter but was significantly elevated in malignant astrocytomas. Malignant astrocytomas also expressed an alternatively spliced form of FGFR-1 (FGFR-1 beta) containing two immunoglobulin-like disulfide loops, whereas normal human adult and fetal brains expressed a receptor form (FGFR-1 alpha) containing three immunoglobulin-like disulfide loops. Intermediate grades of astrocytic tumors exhibited a gradual loss of FGFR-2 and a shift in expression from FGFR-1 alpha to FGFR-1 beta as they progressed from benign to malignant phenotype. These results suggest that differential expression and alternative splicing of FGFRs may be critical in the malignant progression of astrocytic tumors.

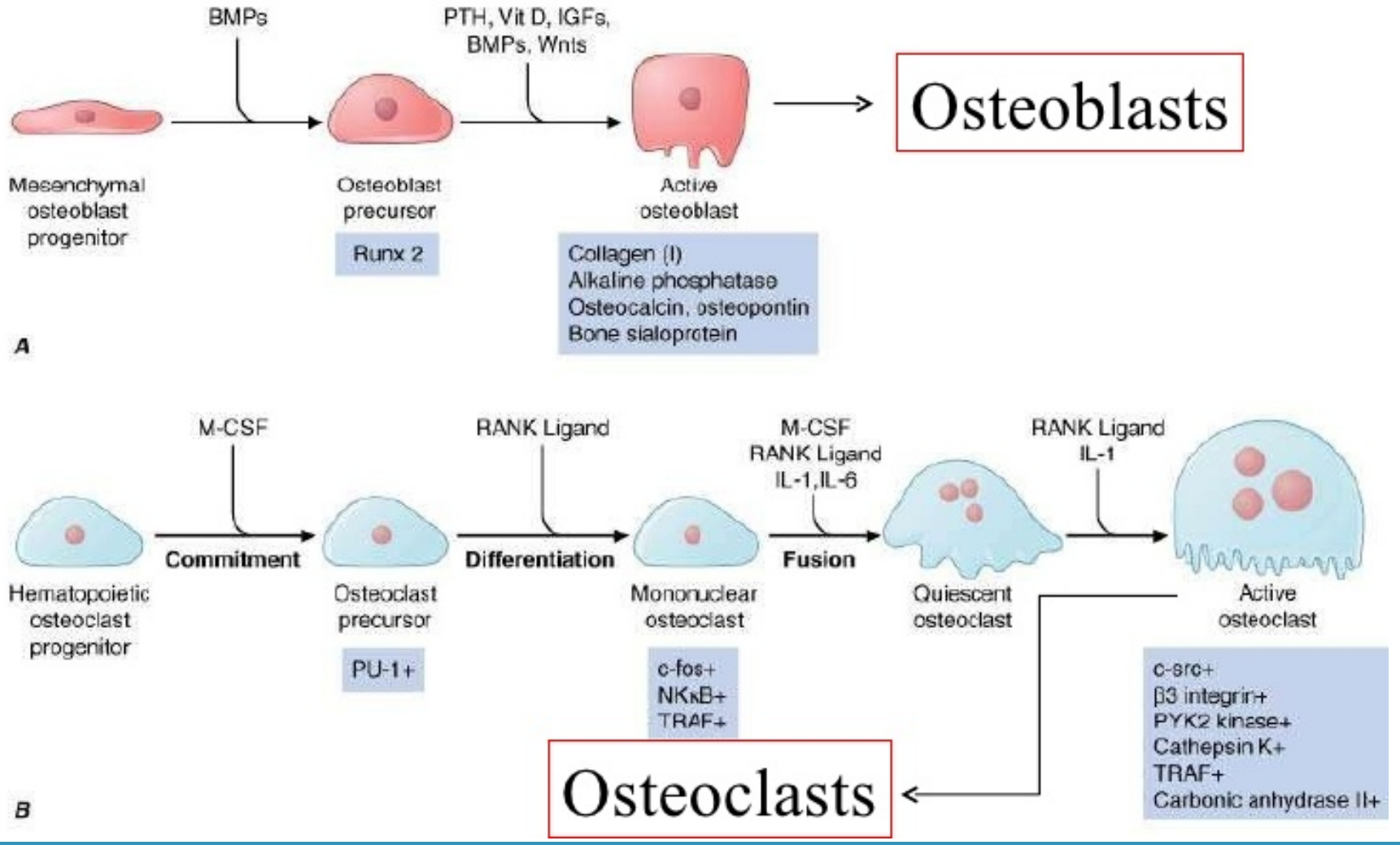
Yamaguchi et al Proc Natl Acad Sci 1994

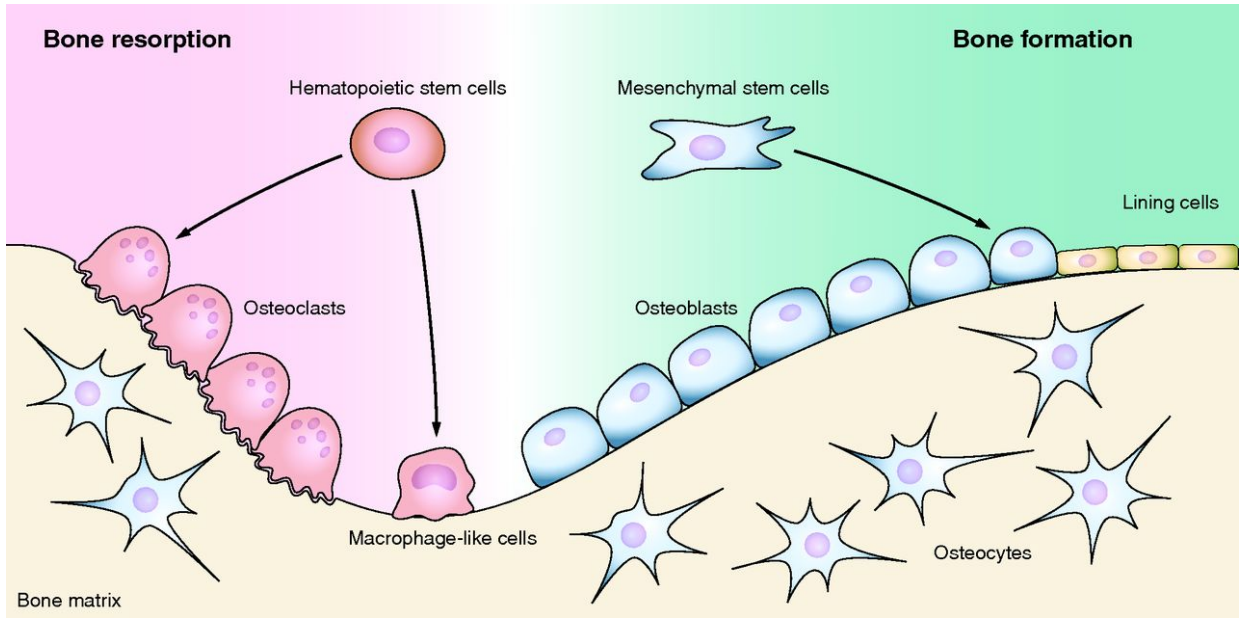
Ligand activation of alternatively spliced fibroblast growth factor receptor-1 modulates pancreatic adenocarcinoma cell malignancy

Pancreatic adenocarcinoma continues to be a devastating tumor (28,000 new cases per year in the United States; 10% 2-year survival). Pancreatic adenocarcinoma frequently (90% of the time) overexpresses fibroblast growth factor ligands (FGF-1 and FGF-2) and alternatively spliced high-affinity receptors (FGFR-1beta) (FGFR-1alpha was previously found in normal pancreatic tissue). To study the significance of this observation in vitro, PANC-1 cells were stably transfected via the pMEXneo vector containing FGFR-1alpha (PANC-1alpha) or FGFR-1beta (PANC-1beta) isoforms. Cells were treated with 1 mg/ml of 5-fluorouracil. Cells were evaluated for growth inhibition, apoptosis (propidium iodide staining and flow cytometry, caspase 3 activation) and for Bcl-x(L)/BAX expression (by Western blot analysis). In vivo, 7 x 10(6) cells of each isoform were injected into nude Balb/c mice for xenograft formation (N = 10). Compared to PANC-1beta (9%) in vitro, 5-fluorouracil-induced death was significantly ($P < 0.05$) increased in PANC-1alpha (20%) at 24 hours. Increased cell death in PANC-1alpha was mediated by activated caspase 3 and was correlated with decreased expression of Bcl-x(L)/BAX. In vivo, PANC-1beta readily demonstrated formation of tumor xenograft at 2 weeks, whereas PANC-1alpha did not form tumors. Alternative splicing of FGFR-1 to the beta isoform appears to correlate with pancreatic adenocarcinoma cell growth in vivo and resistance to chemotherapy. Inhibition of FGFR-1 splicing or overexpression of FGFR-1alpha inhibits pancreatic adenocarcinoma cell growth in vivo and restores cytotoxic responses to chemotherapy, thereby suggesting the basis of rational interventional strategies for this devastating tumor.

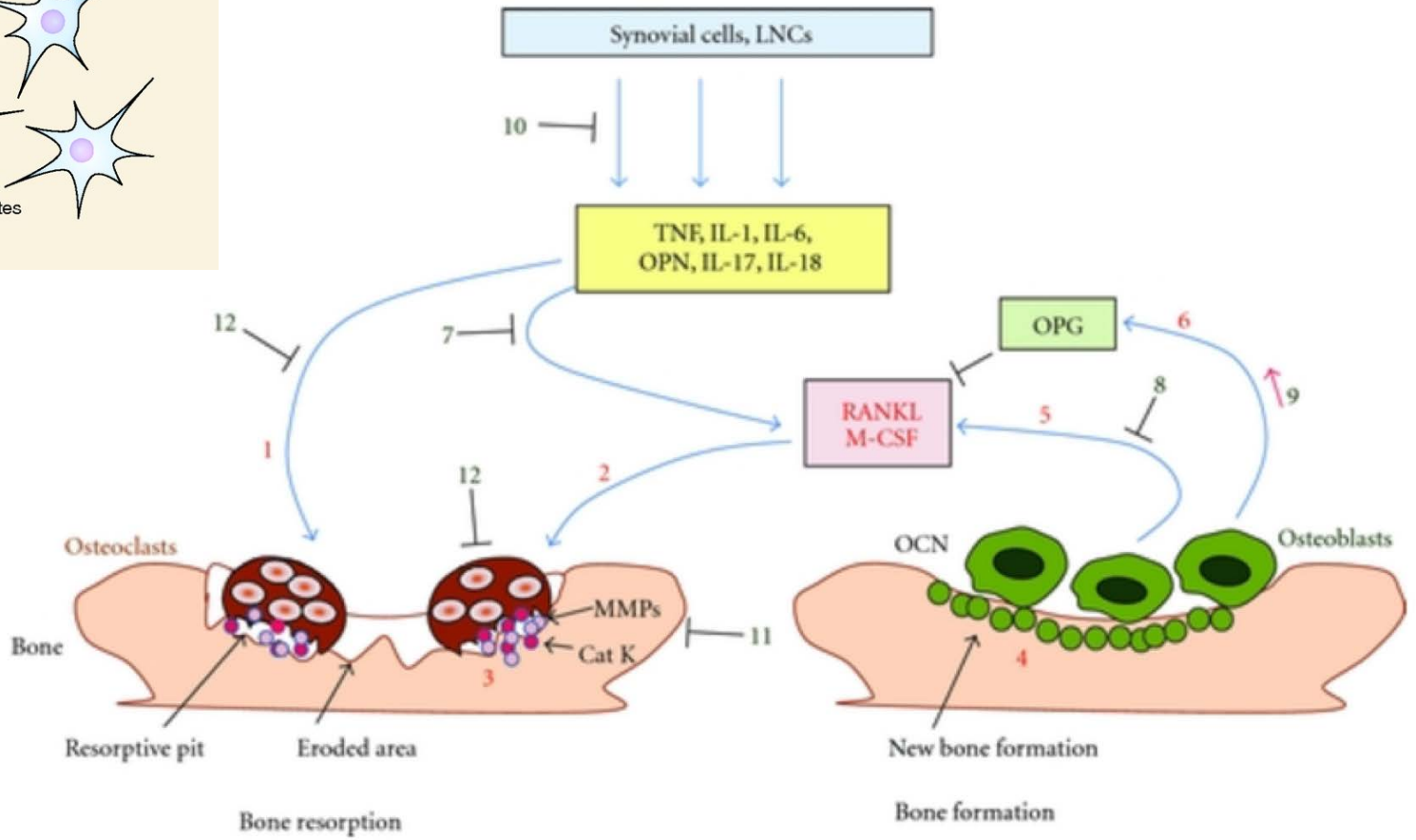
Vickers et al, J Gastrointest Surg 2002

Maturation Pathway



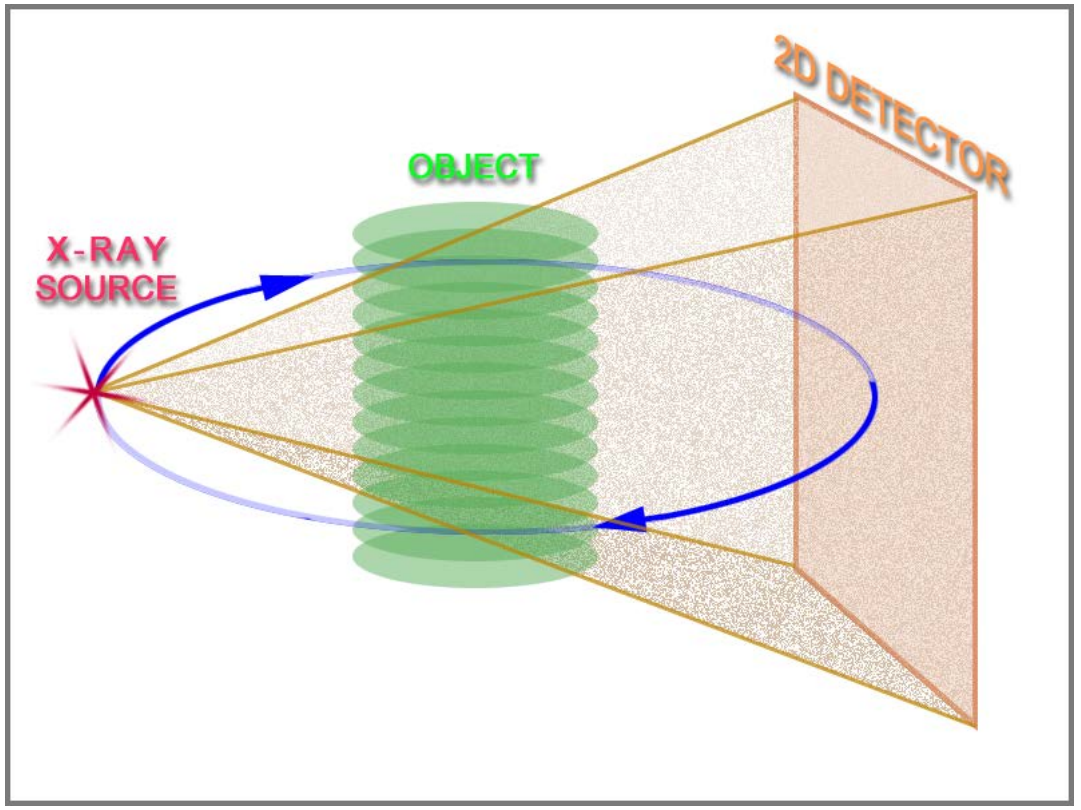

Bone resorption
Bone formation
Hematopoietic stem cells
Mesenchymal stem cells
Lining cells
Osteoclasts
Osteoblasts
Macrophage-like cells
Osteocytes
Bone matrix

Bone


Synovial cells, LNCs
10
TNF, IL-1, IL-6,
OPN, IL-17, IL-18
12
7
OPG
6
9
8
RANKL
M-CSF
5
1
2
12
Osteoclasts
OCN
Osteoblasts
MMPs
Cat K
11
Bone
3
4
Resorptive pit
Eroded area
New bone formation
Bone resorption
Bone formation

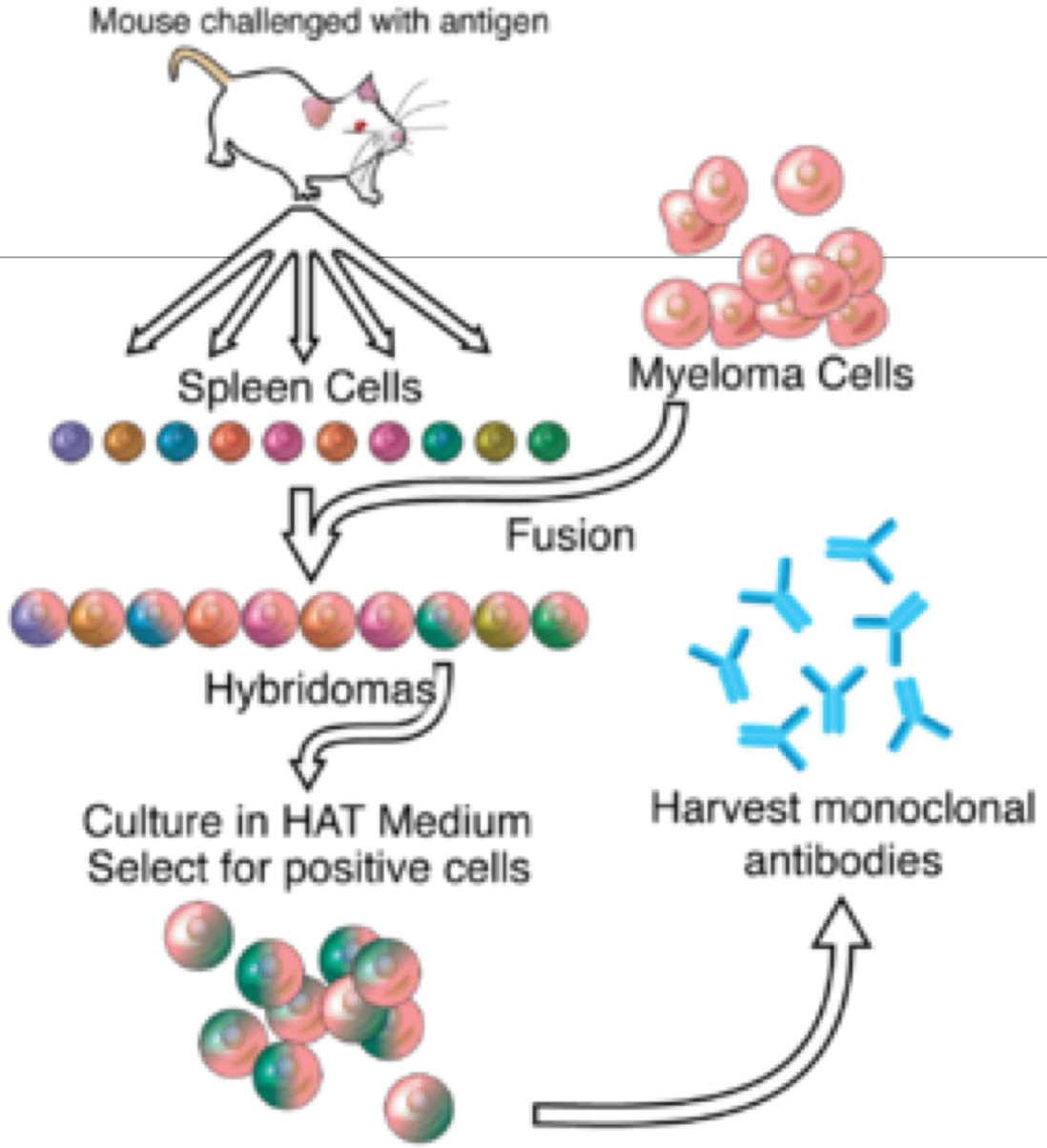
BHM

microCT

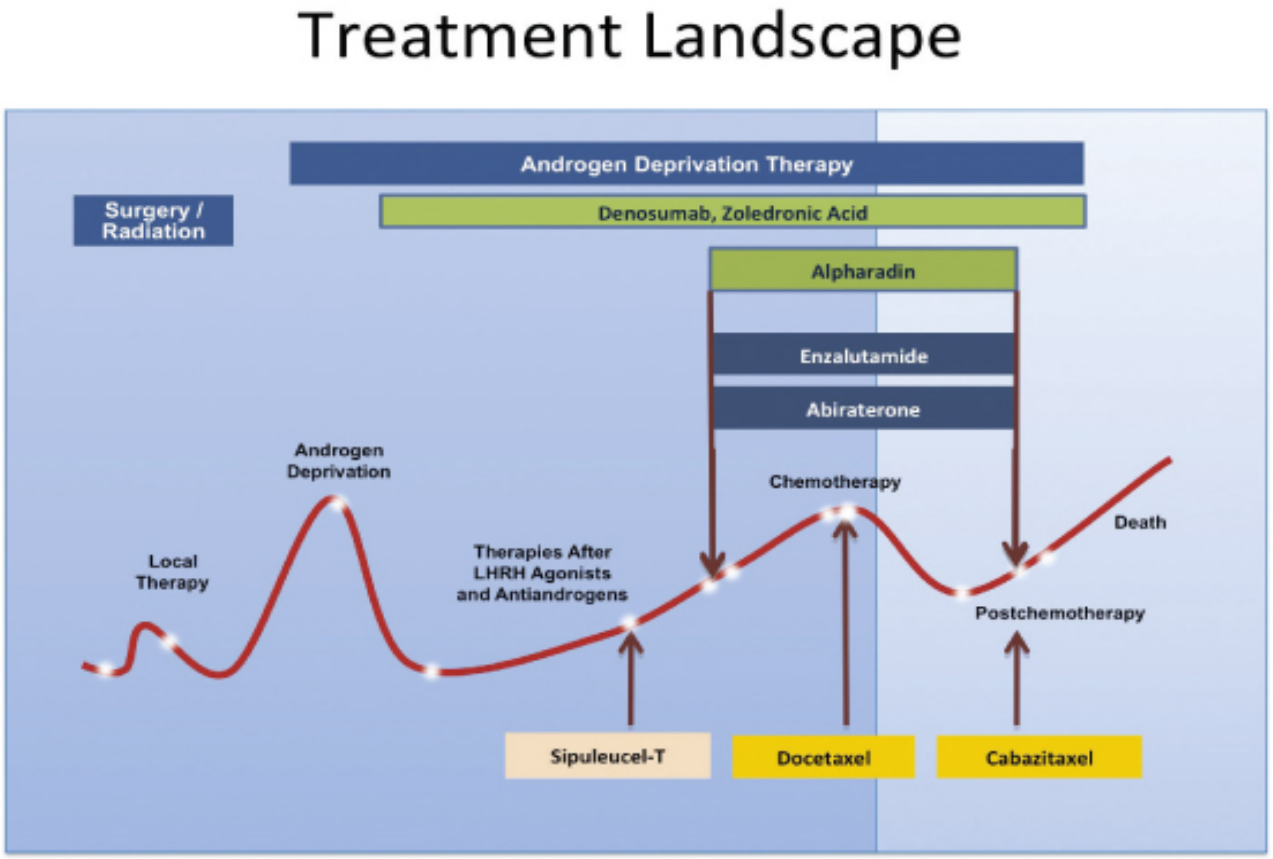


Micro Photonics

Hybridoma technology



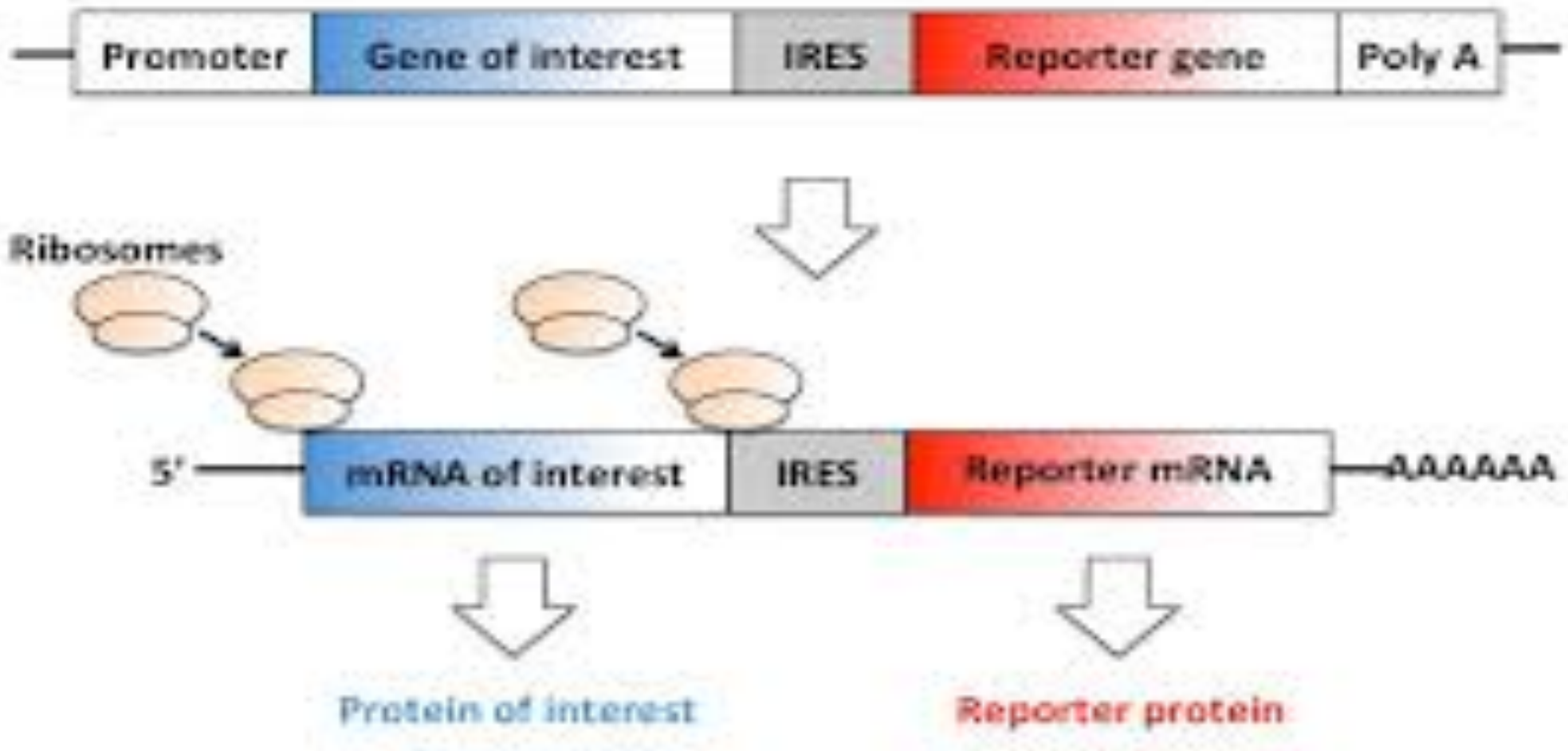
Clinical treatment PCa



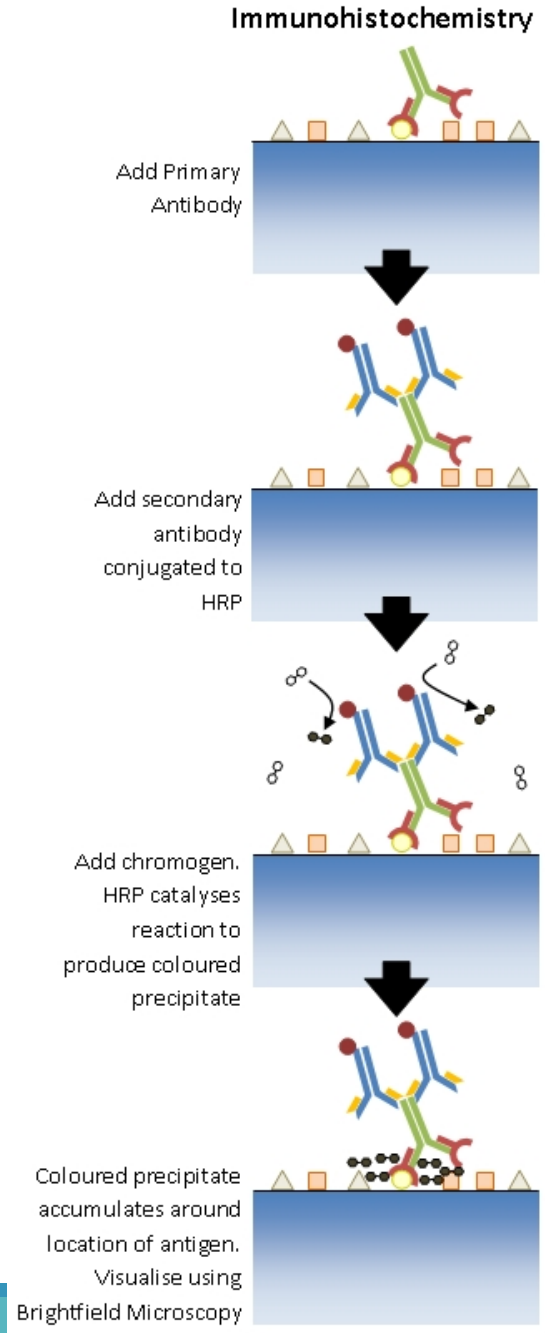
ASCO post, 2012

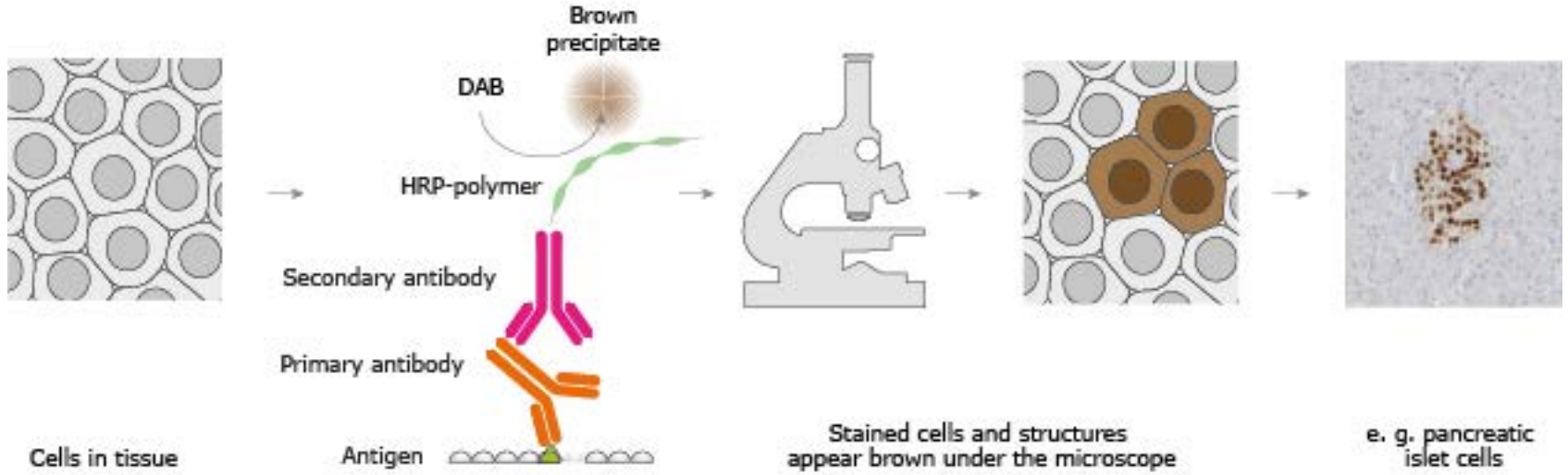
PCa samples

BICISTRONIC



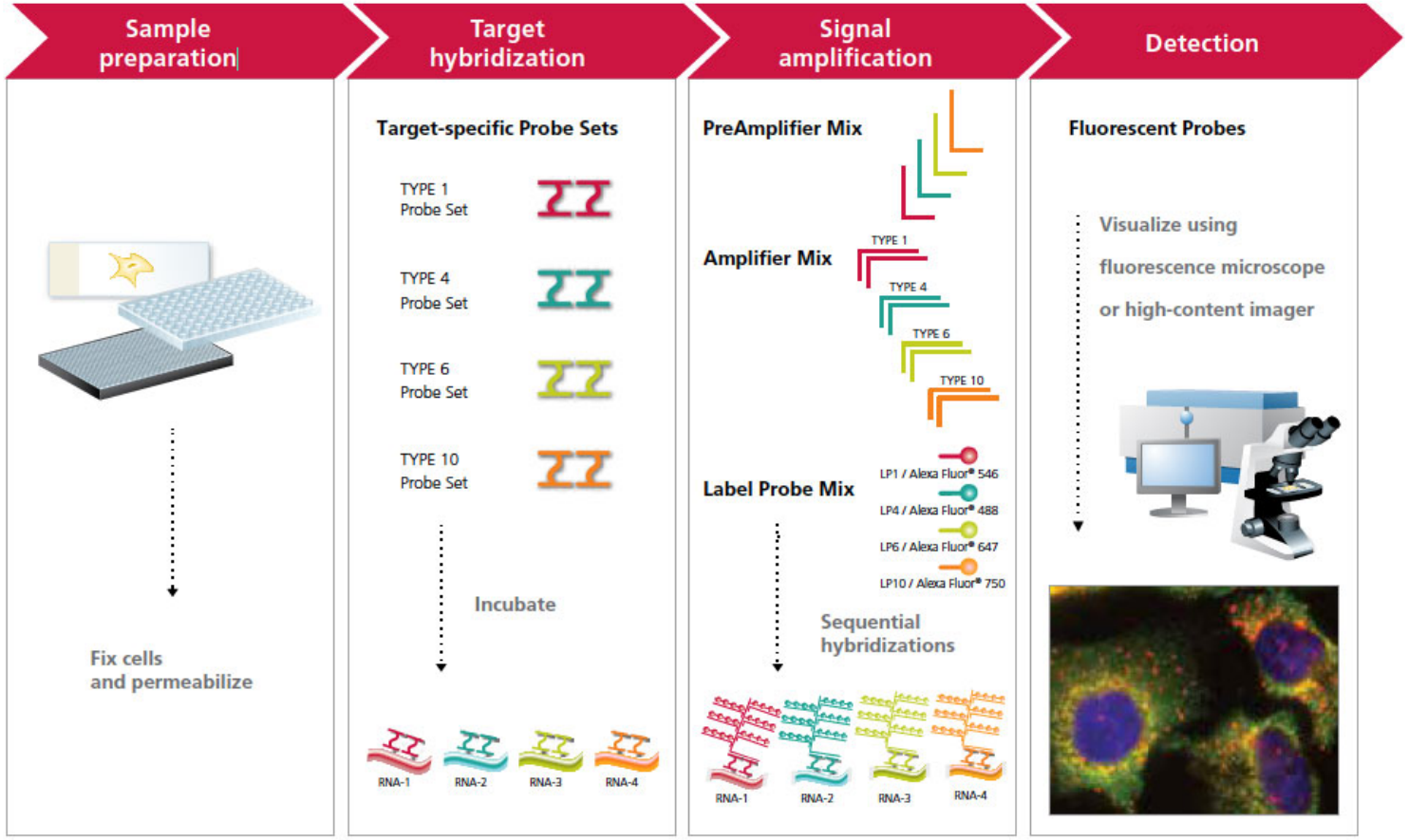
IHC





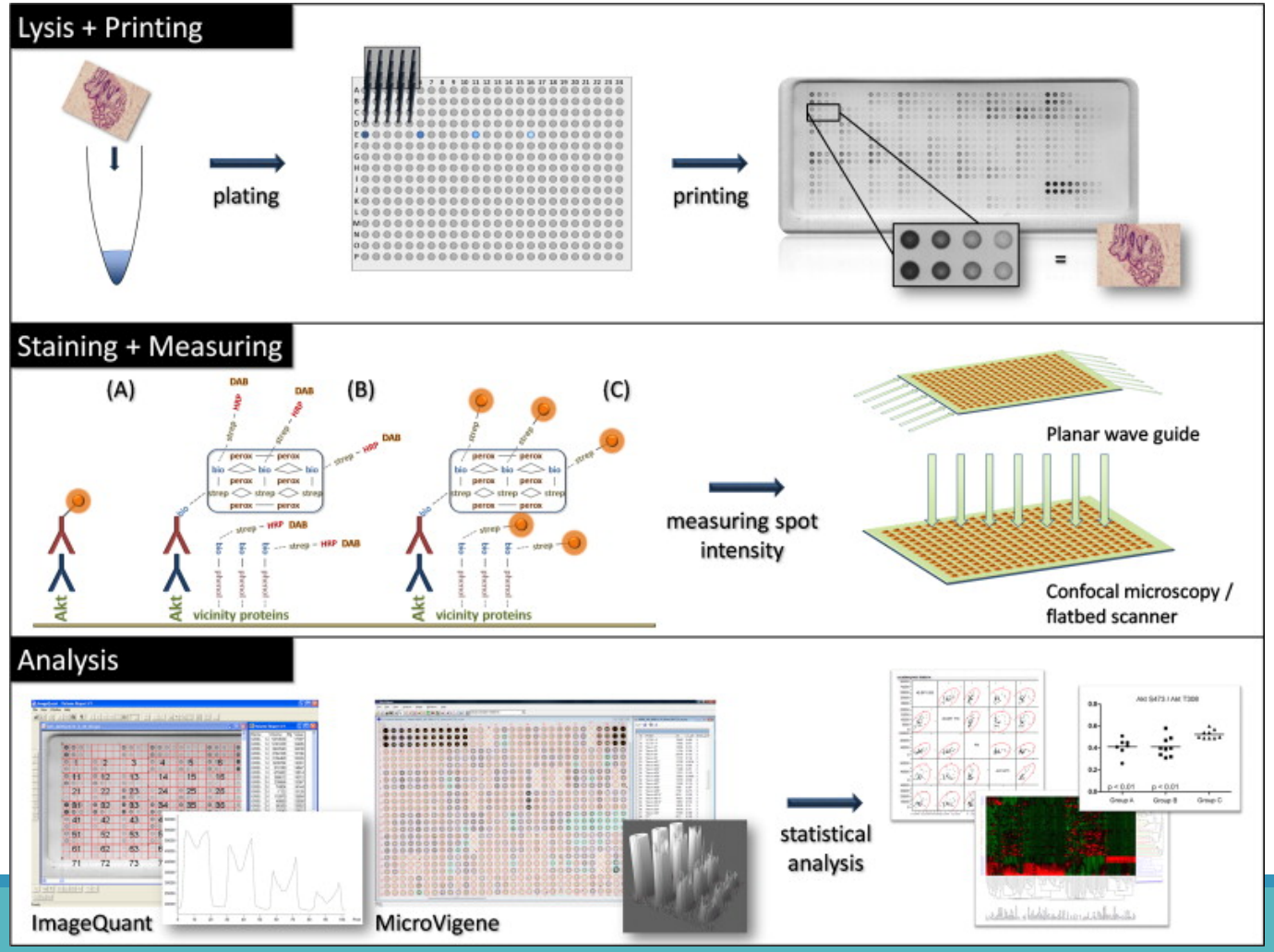
Diamantina Institute, Univ. of Queensland

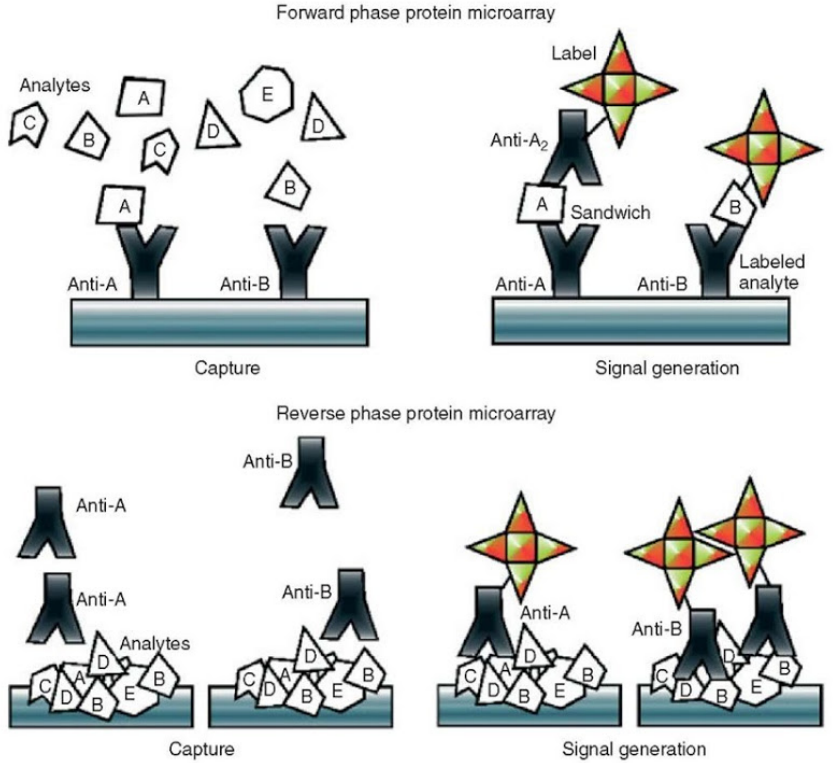
RNA-ISH


Sample preparation
Target hybridization
Signal amplification
Detection
Fix cells and permeabilize
Target-specific Probe Sets
TYPE 1 Probe Set
TYPE 4 Probe Set
TYPE 6 Probe Set
TYPE 10 Probe Set
Incubate
RNA-1
RNA-2
RNA-3
RNA-4
PreAmplifier Mix
Amplifier Mix
TYPE 1
TYPE 4
TYPE 6
TYPE 10
Label Probe Mix
LP1 / Alexa Fluor® 546
LP4 / Alexa Fluor® 488
LP6 / Alexa Fluor® 647
LP10 / Alexa Fluor® 750
Sequential hybridizations
RNA-1
RNA-2
RNA-3
RNA-4
Fluorescent Probes
Visualize using fluorescence microscope or high-content imager

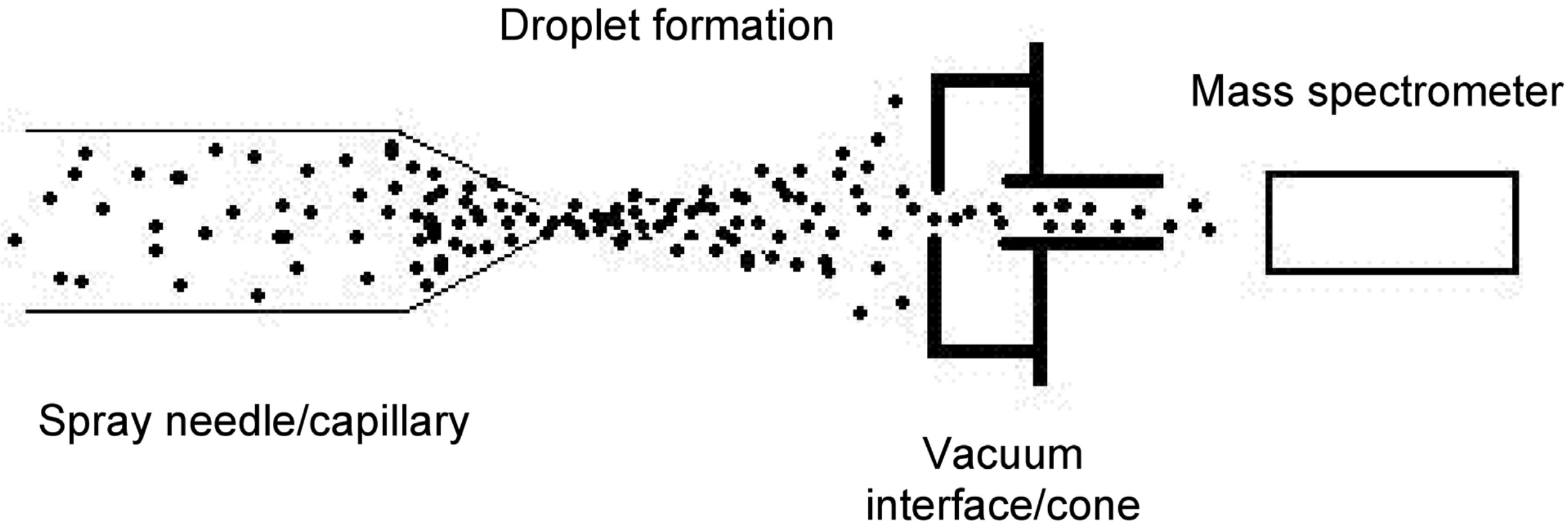
RPPA

In contrast to previous protein arrays that immobilize the probe, our reverse phase protein array immobilizes the whole repertoire of patient proteins that represent the state of individual tissue cell populations undergoing disease transitions (Poweletz et al, Oncogene 2001.

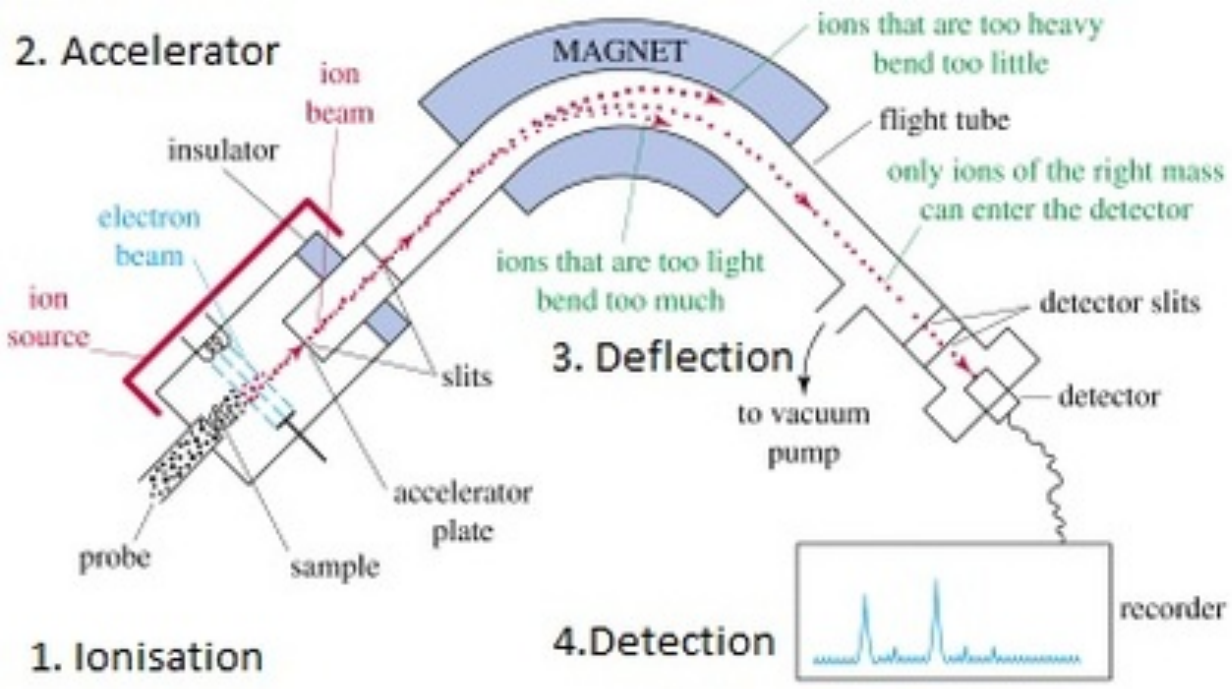


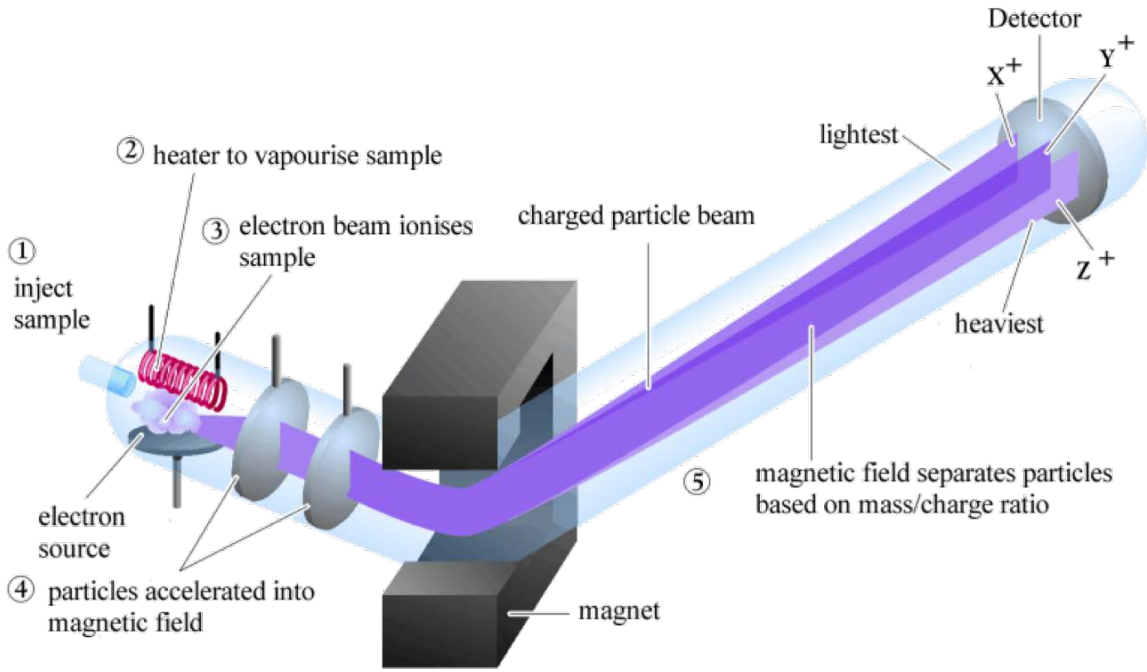


ESI-MS


Droplet formation
Mass spectrometer
Spray needle/capillary
Vacuum
interface/cone

Mass Spec





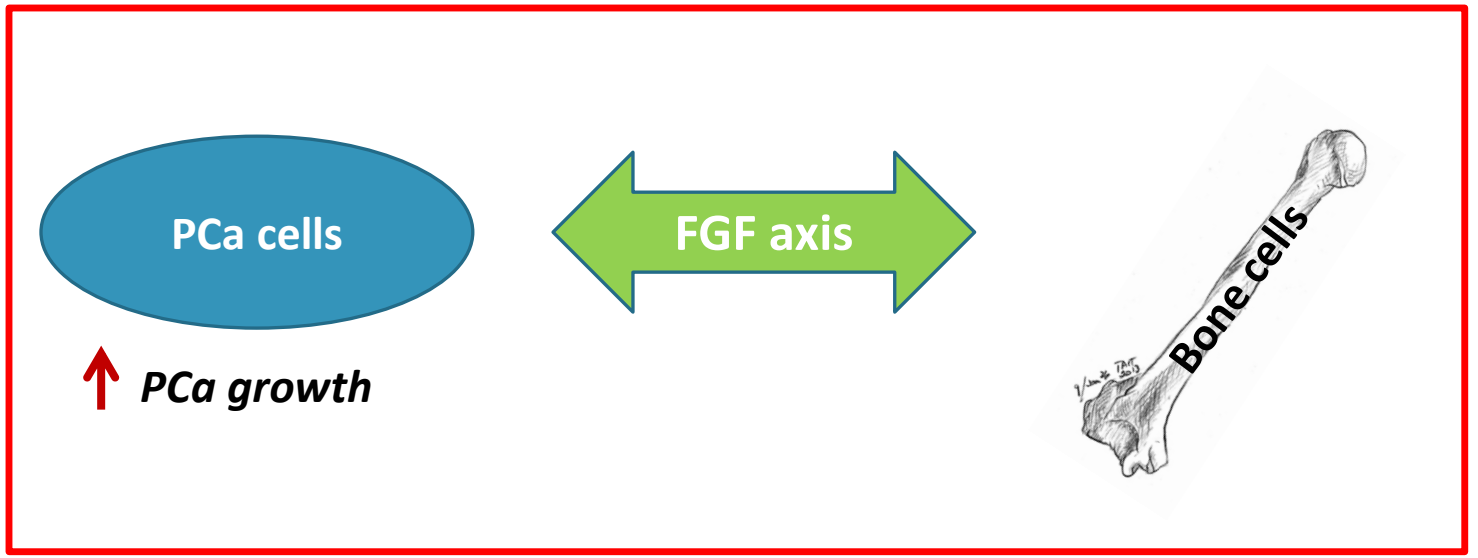
Dept. of Chemistry, Univ. of Calgary

Preliminary data

Previous results from our lab…

FGF axis implicated in PCa bone metastases

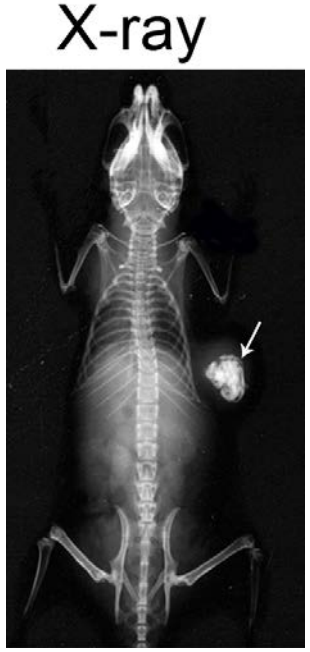
- MDA PCa118 xenografts that induce the ectopic formation of bone ↑FGF9
- FGF9-neutralizing Ab ↓bone tumors
- *FGF9 is expressed in a fraction of advanced human PCas*



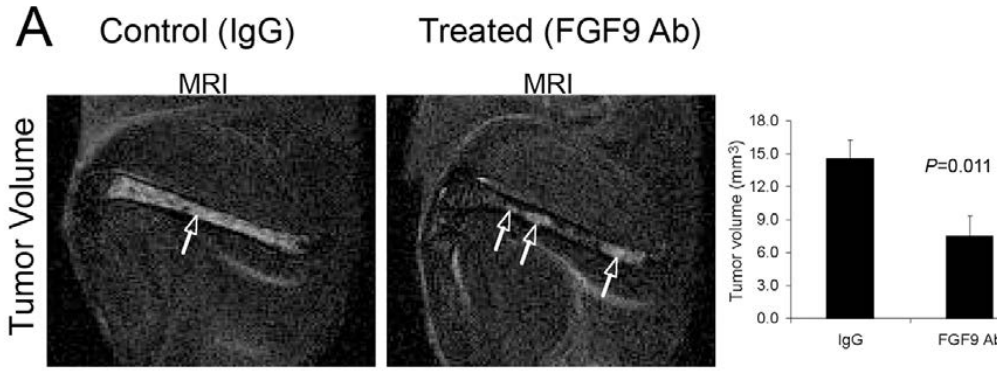
Li et al, 2008 JCI

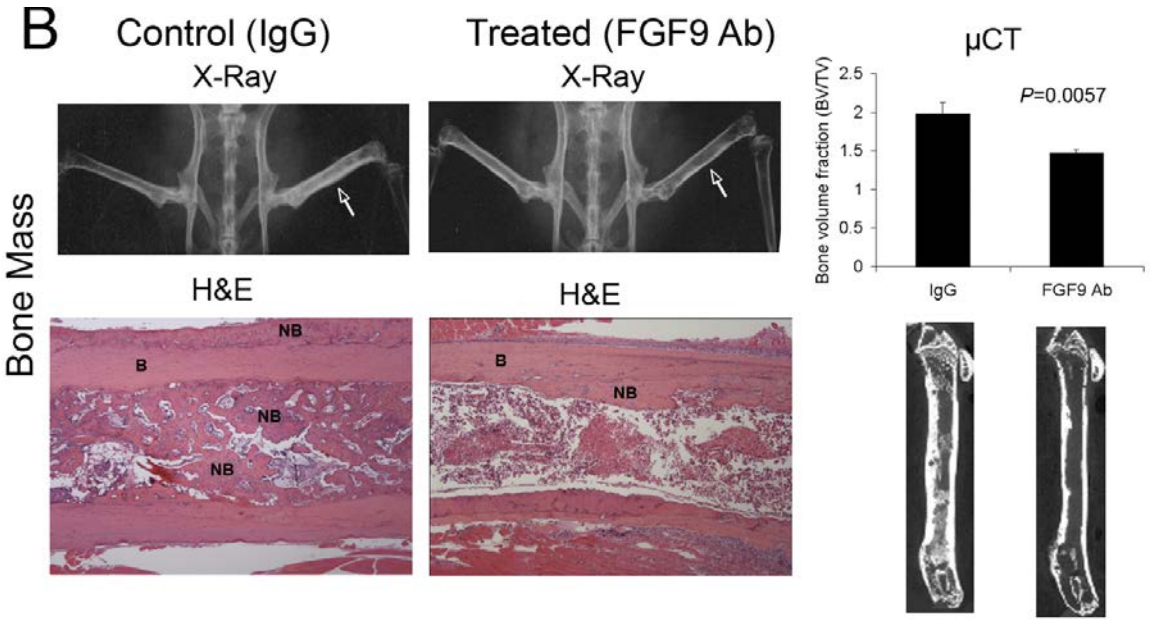
Fibroblast growth factor (FGF) axis in PCa Bone Metastases

Bone metastasis-derived xenograft MDA PCa 118b



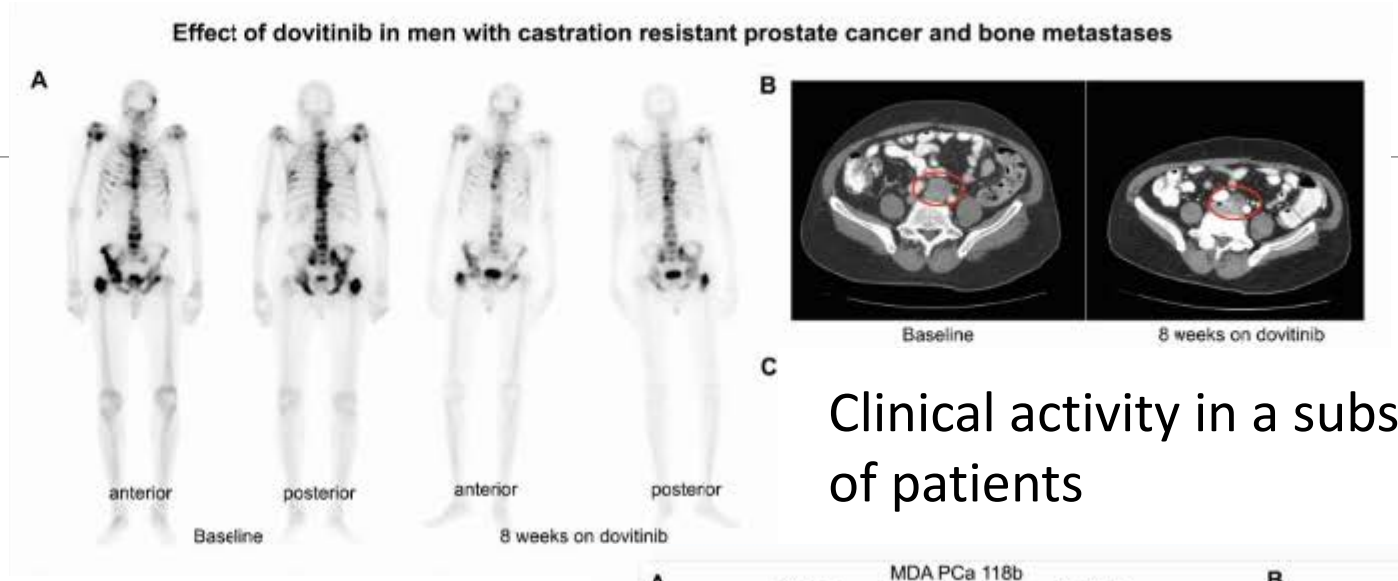
Ectopic bone formation → Gene array analysis → **FGF9**





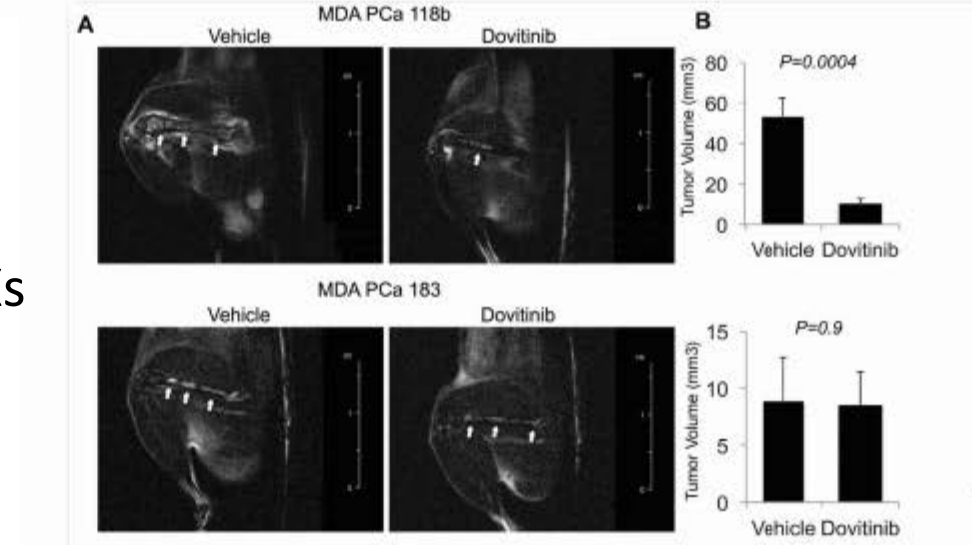
Li et al J Clin Invest 2008

FGFR blockade



Clinical activity in a subset of patients

Antitumor activity in PDXs with high FGFR1



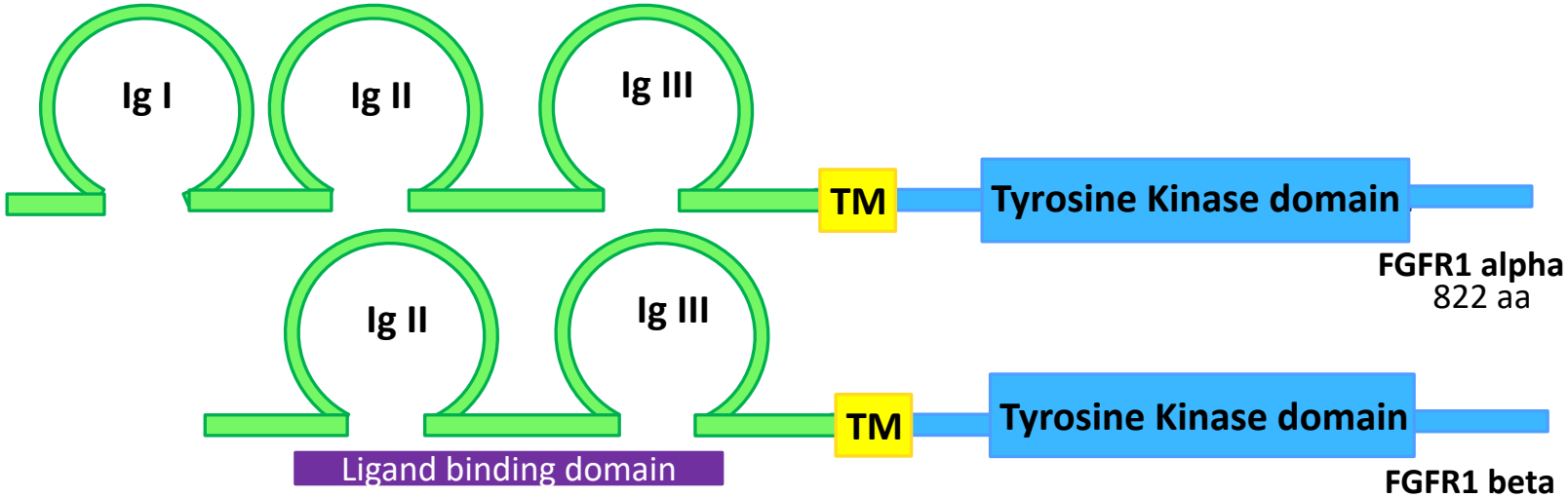
PCa 118b expresses 428 RPKM FGFR1, 3 FGFR2, 8 FGFR3, and 0.8 FGFR4. MDA PCa 183 expresses 32 FGFR1, 0.4 FGFR2, 0.7 FGFR3, and 0.7 FGFR4.

Wan et al, 2014 STM

FGFR1 isoforms RNA-seq

Predicted protein length	Most abundant expressed transcripts
731-733 aa	ENST00000326324 ENST00000356207 ENST00000397103
820-853 aa	ENST00000397091
	ENST00000397108
	ENST00000397113
	ENST00000425967
	ENST00000532791

FGFR1 isoforms



25 31
MWSWKCLLFWAVLVTATLCTARPSPTLPEQAQPWGAPVEVESFLVHPGDL
LQLRCRLRDDVQSINWLRDGVQLAESNRTRITGEEVEVQDSVPADSGLYAC
VTSSPSGSDTTYFSVNVSDALPSSEDDDDDDDSSSEEKETDNTKPNRMPVA
PYWTSPEKMEKKLHAVPAAKTVKFKCPSSGTPNPTLRWLKNGKEFKPDHRI
GGYKVRYATWSIIMDSVVPSDKGNYTCIVENEYGSINHTYQLDVVERSPHRP
ILQAGLPANKTVALGSNVEFMCKVYSDPQPHIQWLKHIEVNGSKIGPDNLPY
VQILKTAGVNTTDKEMEVLHLRNVSFEDAGEYTCLAGNSIGLSHHSAWLTVL
EALEERPAVMTSPLYLEIIIYCTGAFLISCMVGSVIVYKMKSGTKKSDFHSQM
AVHKLAKSIPLRRQVTVSADSSASMNSGVLLVRPSRLSSSGTPMLAGVSEYE
LPEDPRWELPRDRLVLGKPLGEGCFGQVVLAEAIGLDKDKPNRVTKVAVKM
LKSDATEKDLSDLISEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYASKGN
LREYLQARRPPGLEYCYNPSHNPEEQLSSKDLVSCAYQVARGMEYLASKKC
IHRDLAARNVLVTEDNVMKIADFGLARDIHHIDYYKKTTNGRLPVKWMAPEA
LFDRIYTHQSDVWSFGVLLWEIFTLGGSPYPGVPVEELFKLLKEGHRMDKPS
NCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRIVALTSNQEYLDLSMPL
DQYSPSFPDTRSSTCSSGEDSVFSHEPLPEEPCLPRHPAQLANGGLKRR

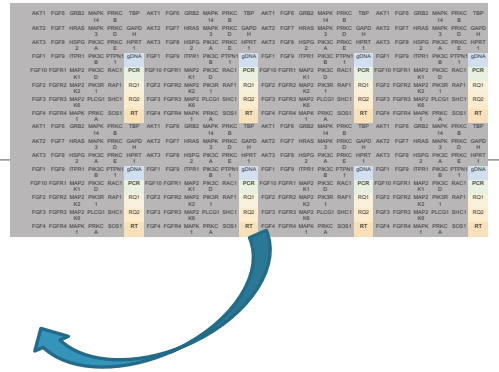
Johnson and Williams, 1993 Adv Cancer Res

FGFR1 isoforms have been associated with pancreatic cancer, breast cancer and glioblastoma (Bruno et al Hum Mol Genet 2004)

1- Assess the signaling pathways activated by FGFR1 in PCa cells

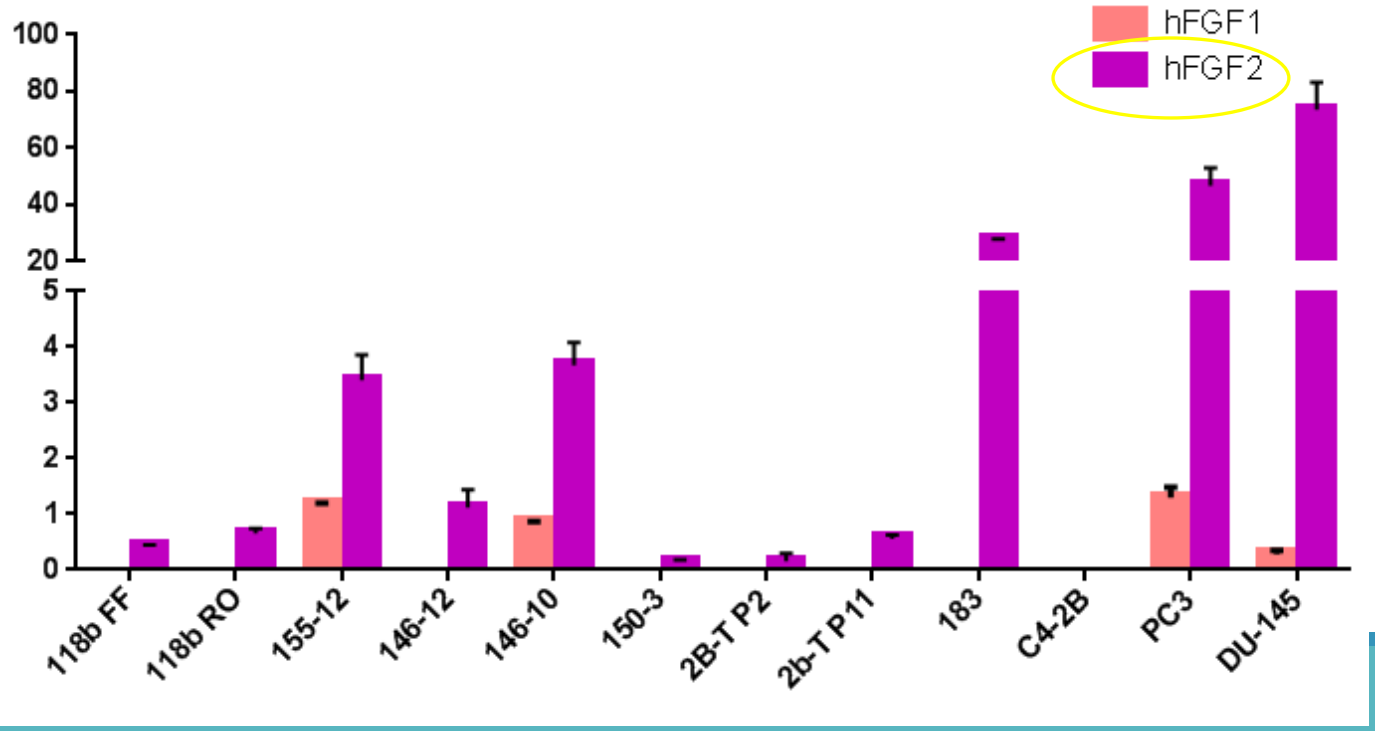
Induce signaling with FGF ligand… Which?

- Screening: **FGF Family Signaling** array in PDX samples



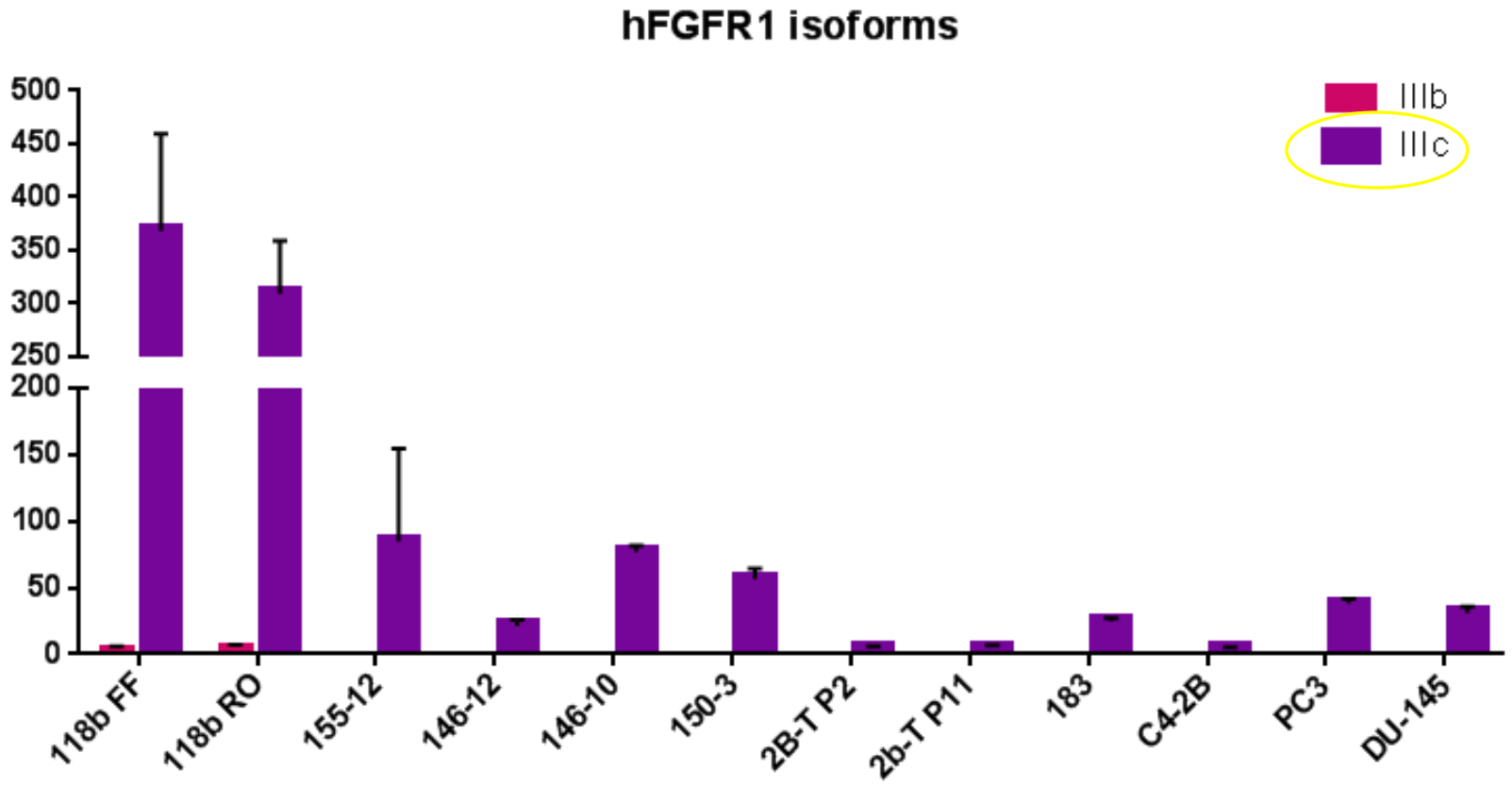
Candidates: FGF1, FGF2, FGF8, FGF9

- Validate with RT-PCR

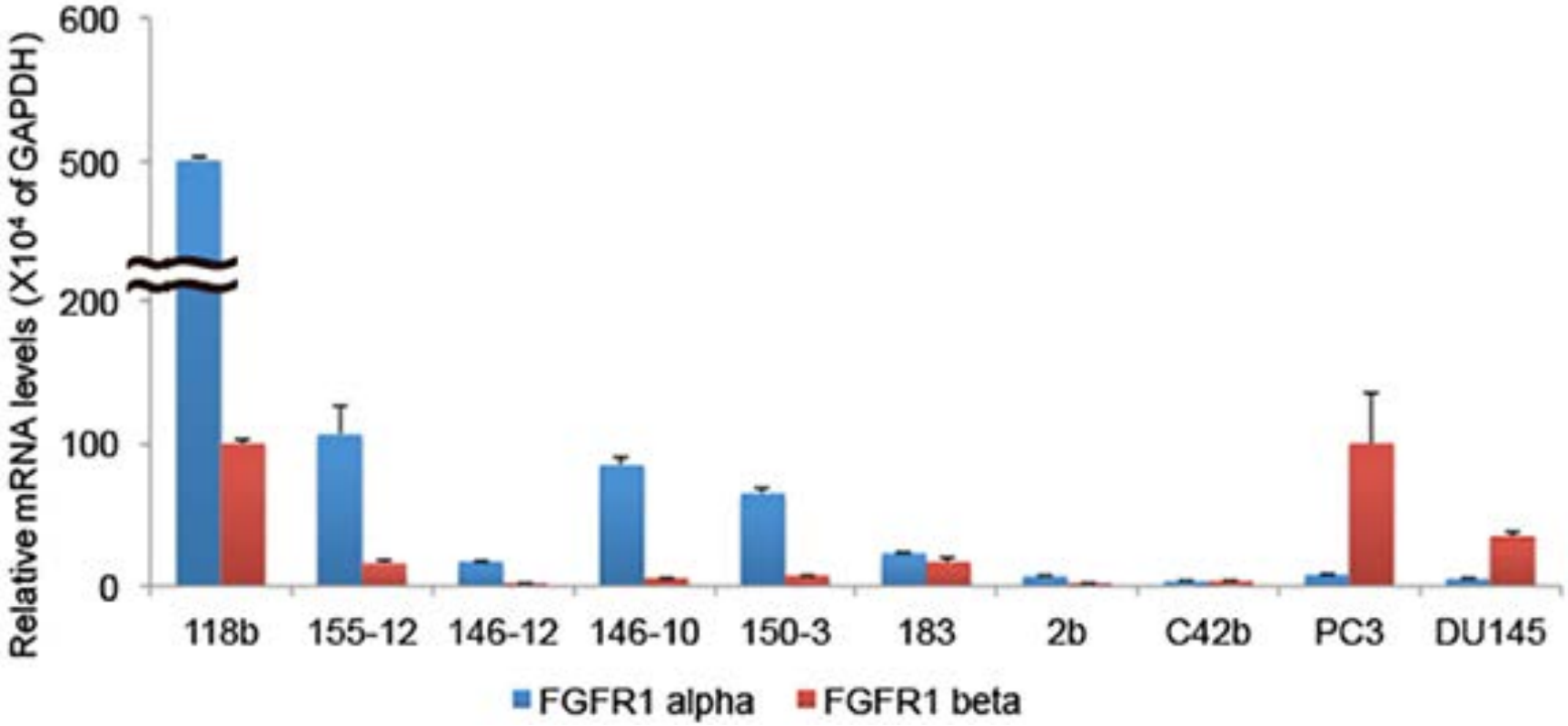


Proposed Work

Validate findings of RNA sequencing by RT-PCR using PDX.



- RT-PCR using PDX and PCa cell lines.

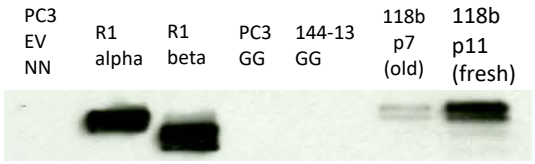


FGFR1 expression comparison in PC3 cells

September 15, 2016

ECL GE

FGFR1
92, 120, 145KDa

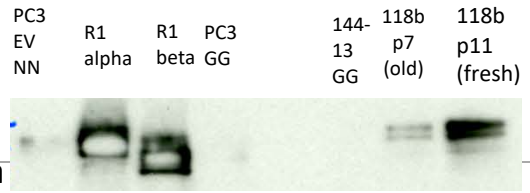


ECL GenDepot

GAPDH
37 KDa

ECL Thermo

FGFR1
92, 120, 145KDa



ECL GenDepot

GAPDH
37 KDa

SuperSignal™ West Femto Maximum Sensitivity Substrate (Gallick's Lab)

*Saturated at 1 sec ET

ET= 30sec

Primary antibody: FGFR1, 1:100 dilution. Cell Signaling Cat# 9740
Primary antibody: GAPDH, 1:500 dilution. Cell Signaling Cat# 2118
Secondary antibody: anti rabbit IgG, 1: 2000 dilution Cat# 7074 Cell Signaling

Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

b. Mine TCGA for FGFR1 isoform data

The American Journal of Pathology, Vol. 178, No. 4, April 2011

DOI: 10.1016/j.ajpath.2010.12.046

Tumorigenesis and Neoplastic Progression

The Androgen-Regulated Calcium-Activated Nucleotidase 1 (CANT1) Is Commonly Overexpressed in Prostate Cancer and Is Tumor-Biologically Relevant *in Vitro*

Oncogene (2015) **34,** 3744–3750; doi:10.1038/onc.2014.307; published online 22 September 2014

UAP1 is overexpressed in prostate cancer and is protective against inhibitors of *N*-linked glycosylation

20 genes most and least correlated with FGFR1 splice score					
Highest correlation (alpha)			Lowest correlation (beta)		
gene	correlation	coefficient	gene	correlation	coefficient
PLEKHH1	0.3760326	2.5259998	PMP22	-0.5641646	-3.928847
THTPA	0.3744982	1.0365405	CORO1C	-0.5611298	-2.550176
SLC25A42	0.3651624	1.6936021	SERPING1	-0.5593268	-4.199745
PSD4	0.3630576	1.2315951	FBLN5	-0.5554176	-4.084689
CANT1	0.3605198	1.3428722	C1S	-0.5541675	-4.465541
LANCL2	0.3472923	0.8219091	GLT8D2	-0.5520242	-3.920179
SPTBN2	0.3386063	1.5285074	SYNPO	-0.5486689	-3.730633
SLC35E1	0.3309034	1.0048222	IGFBP7	-0.5479488	-3.541869
CNNM3	0.3287317	0.8415453	RAB31	-0.5466158	-3.504471
ATP13A2	0.3279984	1.0954338	TNFAIP8L3	-0.5452566	-4.440794
KIAA0319L	0.3274587	1.1546841	RFTN1	-0.5434276	-3.650135
C15orf37	0.3265595	1.5139643	A2M	-0.5428293	-4.033372
ALG6	0.3260368	1.2212658	CTSK	-0.539713	-3.846549
CREB3L4	0.3243941	1.3629656	C3orf59	-0.5336978	-3.347806
TTLL12	0.3242178	1.2114275	TIMP2	-0.5324241	-3.359562
INTS5	0.3241892	0.7226837	C1R	-0.5310479	-4.142481
MOGS	0.3239719	0.8849534	LHFP	-0.5270556	-3.14607
LOC401588	0.3189179	1.2685184	CLIC2	-0.5267913	-3.077692
UAP1	0.3173437	1.6361159	CALHM2	-0.526377	-2.984523
KIAA1543	0.3169949	1.1838213	MFAP4	-0.5261008	-4.593608

UAP1 and CANT1 Prostate Cancer

EBioMedicine. 2016 Jun;8:103-16. doi: 10.1016/j.ebiom.2016.04.018. Epub 2016 Apr 20.

Glycosylation is an Androgen-Regulated Process Essential for Prostate Cancer Cell Viability.

Munkley J[1], Vodak D[2], Livermore KE[3], James K[4], Wilson BT[5], Knight B[6], Mccullagh P[7], Mcgrath J[8], Crundwell M[9], Harries LW[10], Leung HY[11], Robson CN[1] Mills IG[13], Rajan P[14], Elliott DJ[3].

Author information

Abstract

Steroid androgen hormones play a key role in the progression and treatment of prostate cancer, with androgen deprivation therapy be the first-line treatment used to control cancer growth. Here we apply a novel search strategy to identify androgen-regulated cellular pathways that may be clinically important in prostate cancer. Using RNASeq data, we searched for genes that showed reciprocal changes in expression in response to acute androgen stimulation in culture, and androgen deprivation in patients with prostate cancer Amongst 700 genes displaying reciprocal expression patterns we observed a significant enrichment in the cellular process glycosylati Of 31 reciprocally-regulated glycosylation enzymes, a set of 8 (GALNT7, ST6GalNAc1, GCNT1, UAP1, PGM3, CSGALNACT1, ST6GAL1 and EDEM3) were significantly up-regulated in clinical prostate carcinoma. Androgen exposure stimulated synthesis of glycan structures downstream of this core set of regulated enzymes including sialyl-Tn (sTn), sialyl Lewis(X) (SLe(X)), O-GlcNAc and chondroitin sulphate, suggesting androgen regulation of the core set of enzymes controls key steps in glycan synthesis. Expression of each of these enzymes also contributed to prostate cancer cell viability. This study identifies glycosylation as a global target for androgen control, and suggests loss of specific glycosylation enzymes might contribute to tumour regression following androgen depletion therapy.

UroToday International Journal

www.urotodayinternationaljournal.com
Volume 2 - August 2009

A Four-Gene Expression Signature for Prostate Cancer Cells Consisting of UAP1, PDLIM5, IMPDH2, and HSPD1

Isabelle Guyon,[1] Herbert A. Fritsche,[2] Paul Choppa,[3] Li-Ying Yang,[2] Stephen D. Barnhill[1]
[1]Health Discovery Corporation, Savannah, Georgia; [2]University of Texas, M.D. Anderson Cancer Center, Houston, Texas;
[3]Clarient Inc., Aliso Viejo, California
Submitted May 19, 2009 - Accepted for Publication June 30, 2009

Cancer Res. 2008 May 1;68(9):3094-8. doi: 10.1158/0008-5472.CAN-08-0198.

Two unique novel prostate-specific and androgen-regulated fusion partners of ETV4 in prostate cancer.

Hermans KG[1], Bressers AA, van der Korput HA, Dits NF, Jenster G, Trapman J.

Author information

Abstract

Recently, fusion of ERG to the androgen-regulated, prostate-specific TMPRSS2 gene has been identified as the most frequent genetic alteration in prostate cancer. At low frequency, TMPRSS2-ETV1 and TMPRSS2-ETV4 fusion genes have been described. In this study, we report two novel ETV4 fusion genes in prostate cancer: KLK2-ETV4 and CANT1-ETV4. Both gene fusions have important unique aspects. KLK2 is a well-established androgen-induced and prostate-specific gene. Fusion of KLK2 to ETV4 results in the generation of an additional ETV4 exon, denoted exon 4a. This novel exon delivers an ATG for the longest open reading frame, in this way avoiding translation start in KLK2 exon 1. Although wild-type CANT1 has two alternative first exons (exons 1 and 1a), only exon 1a was detected in CANT1-ETV4 fusion transcripts. We show that CANT1 transcripts starting at exon 1a have an androgen-induced and prostate-specific expression pattern, whereas CANT1 transcripts starting at exon 1 are not prostate specific. So, the two novel ETV4 fusion partners possess as predominant common characteristics androgen-induction and prostate-specific expression.

Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

b. Mine TCGA for FGFR1 isoform data

Pathways associated to FGFR1 splice score	
alpha	**beta**
mRNA processing	Cross-presentation of soluble exogenous antigens (endosomes)
Processing of Capped Intron-Containing Pre-mRNA	Antigen Processing-Cross presentation
late phase of HIV life cycle	Class I MHC mediated antigen processing & presentation
metabolism of non-coding RNA	Antigen processing: Ubiquitination & Proteasome degradation
NEP/NS2 Interacts with the Cellular Export Machinery	Host Interactions of HIV factors
transport of ribonucleoproteins into the host nucleus	HIV infection
transport of mature transcript to cytoplasm	PDGFRB pathway
Transport of Mature mRNA derived from an Intron-Containing Transcript	Fc gamma R-mediated phagocytosis
RNA Polymerase I Transcription Initiation	Signaling by the B Cell Receptor (BCR)
nucleotide excision repair	Alpha Synulecin Pathway
formation of transcription coupled NER pre-incision complex	developmental biology
Transcription-Coupled Nucleotide Excision Repair (TC-NER)	axon guidance
Mitochondrial tRNA aminoacylation	signaling by NGF
Aminoacyl tRNA biosynthesis	signaling by PDGF
tRNA aminoacylation	HDAC class II pathway
terpenoid backbone biosynthesis	PI3K PLC TRK pathway
cholesterol biosynthesis	hemostasis
Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S	integrin A4B1 pathway
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	endocytosis
protein export	HIF2 pathway
metabolism of polyamines	Innate immune system
Glyoxylate and dicarboxylate metabolism	ILK pathway
	P53 hypoxia pathway
	SNARE interactions in vesicular transport
	regulation of actin cytoskeleton
	(st) integrin signaling pathway
	ARF6 trafficking pathway
	adherens junction
	Pathways in cancer
	acute myeloid leukemia

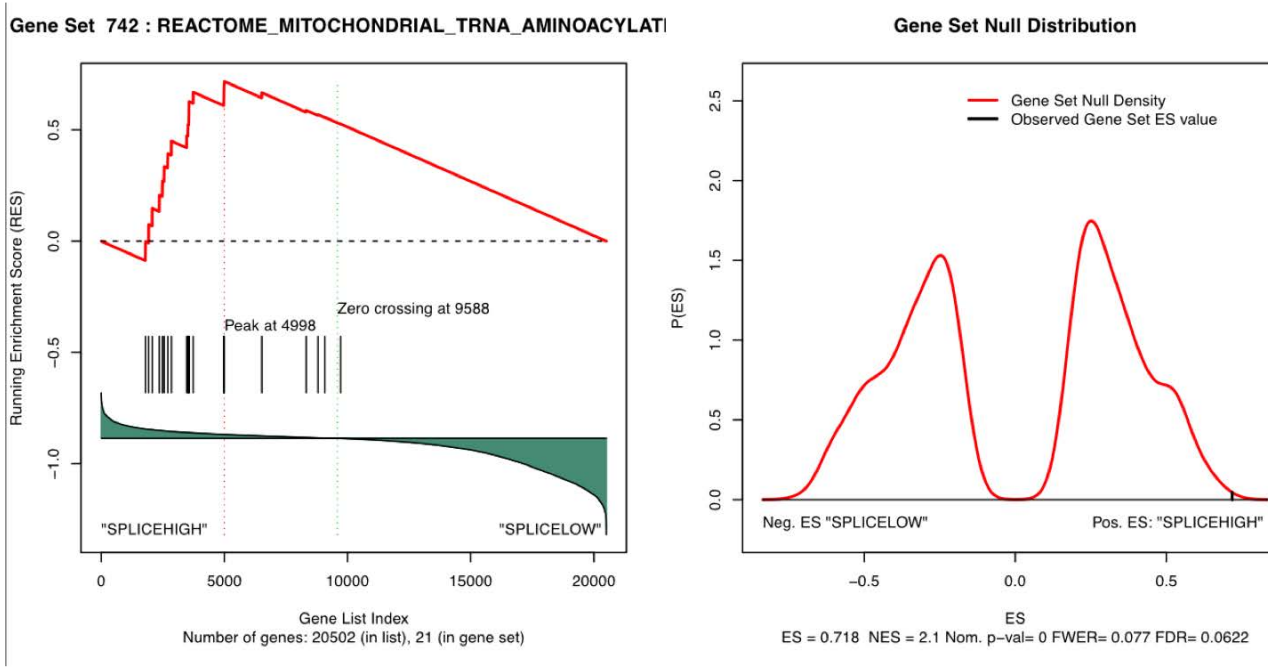
Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

b. Mine TCGA for FGFR1 isoform data

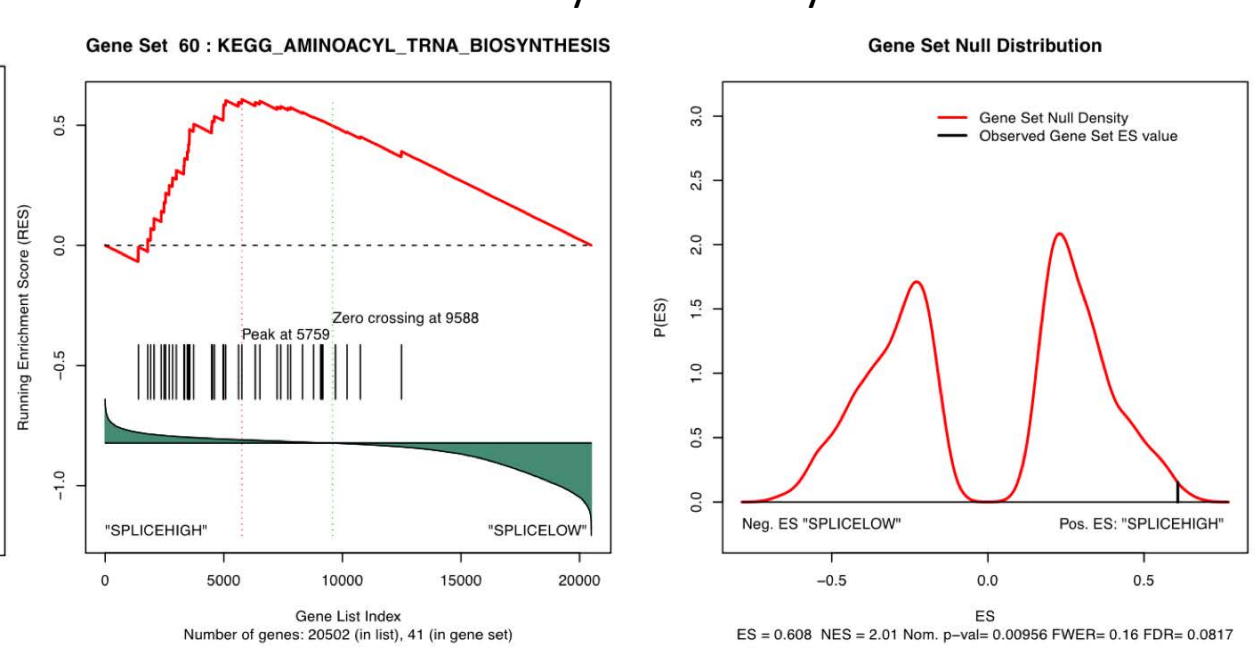
Pathways associated to FGFR1 splice score with highest *P*-value and empirical *P*-value

alpha

Mithocondrial tRNA aminoacylation



Aminoacyl tRNA biosynthesis



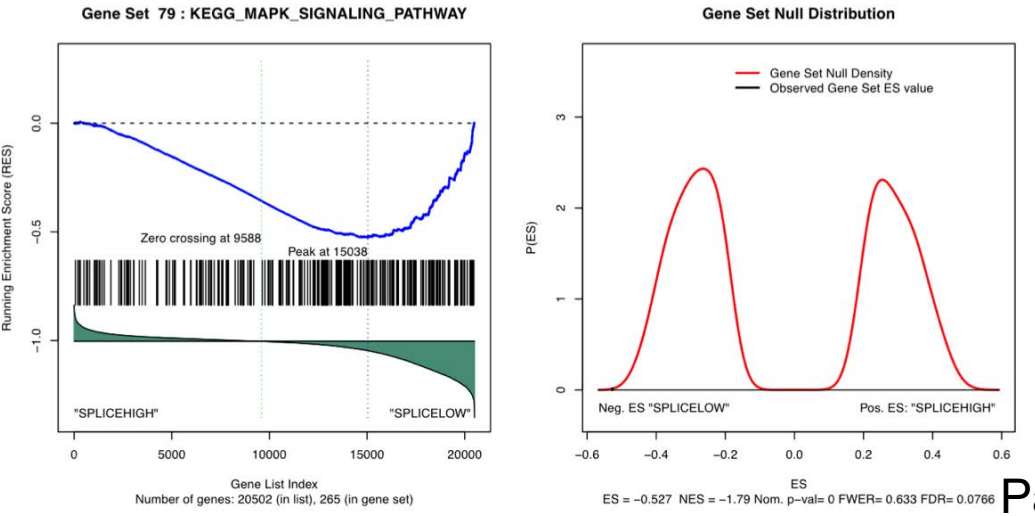
Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

b. Mine TCGA for FGFR1 isoform data

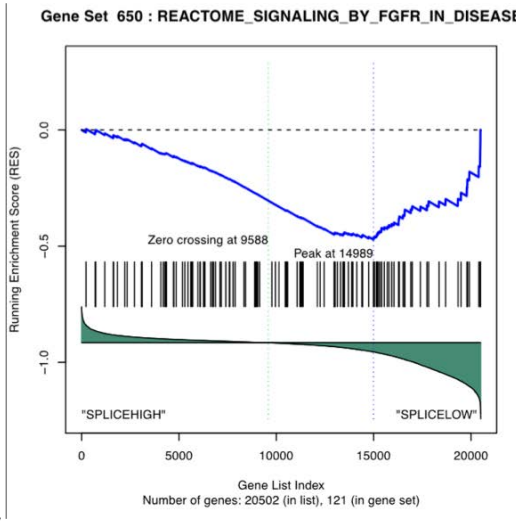
Pathways associated to FGFR1 splice score with highest *P*-value and empirical *P*-value

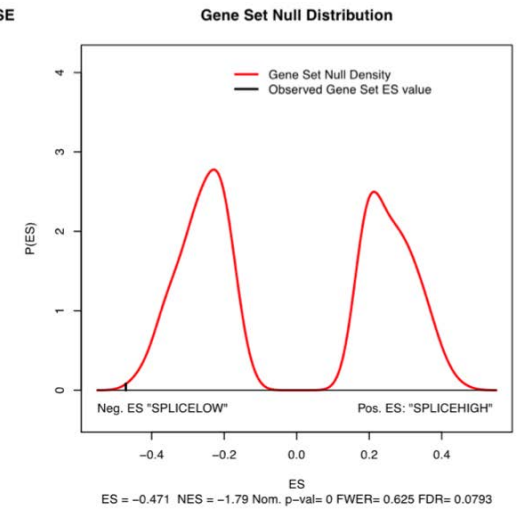
beta → Many pathways associated

MAPK signaling

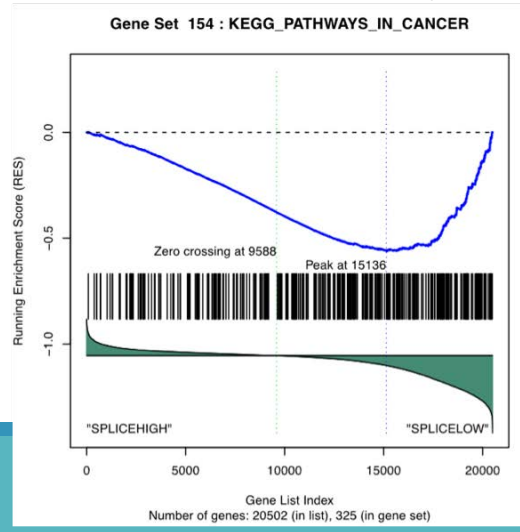


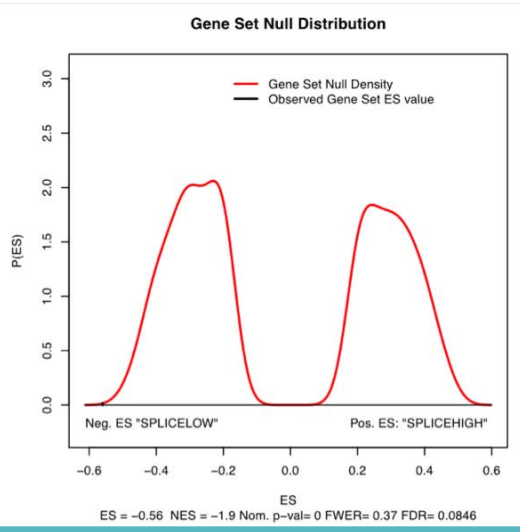
Signaling by FGFR in disease



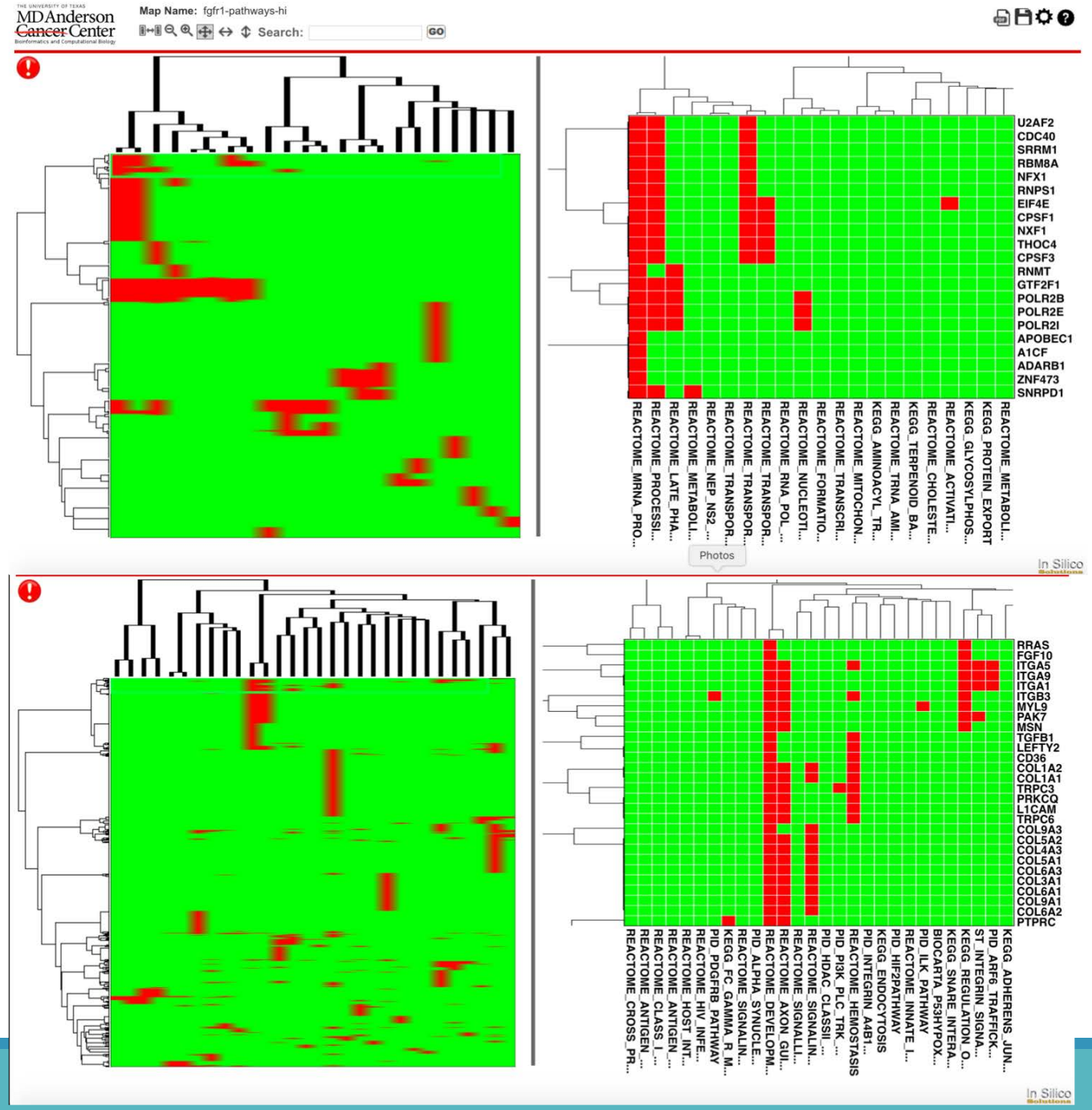


Pathways in cancer



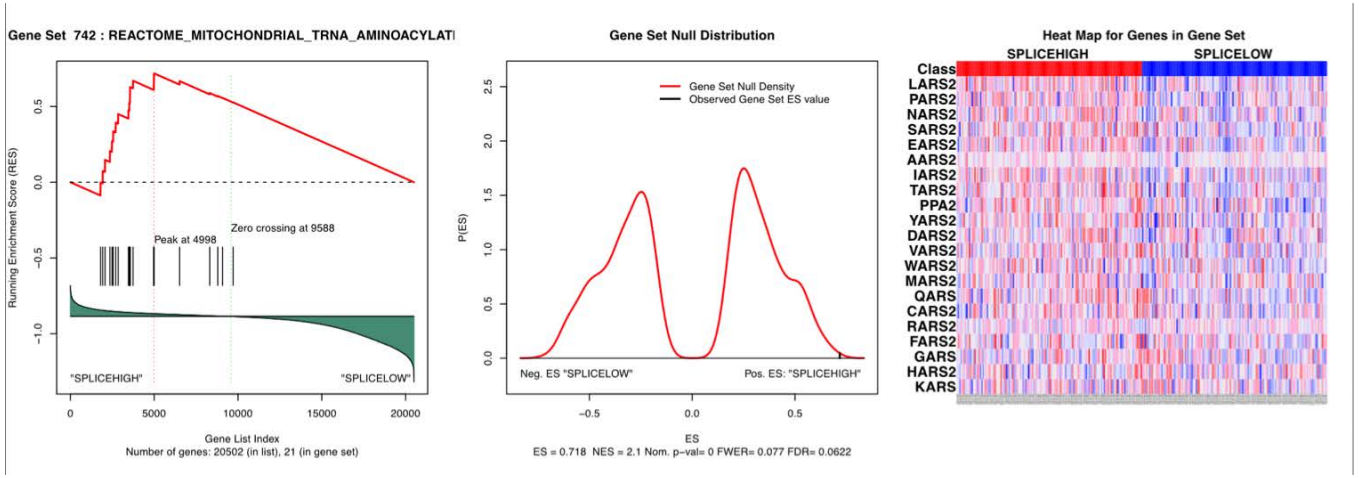


Pathways associated

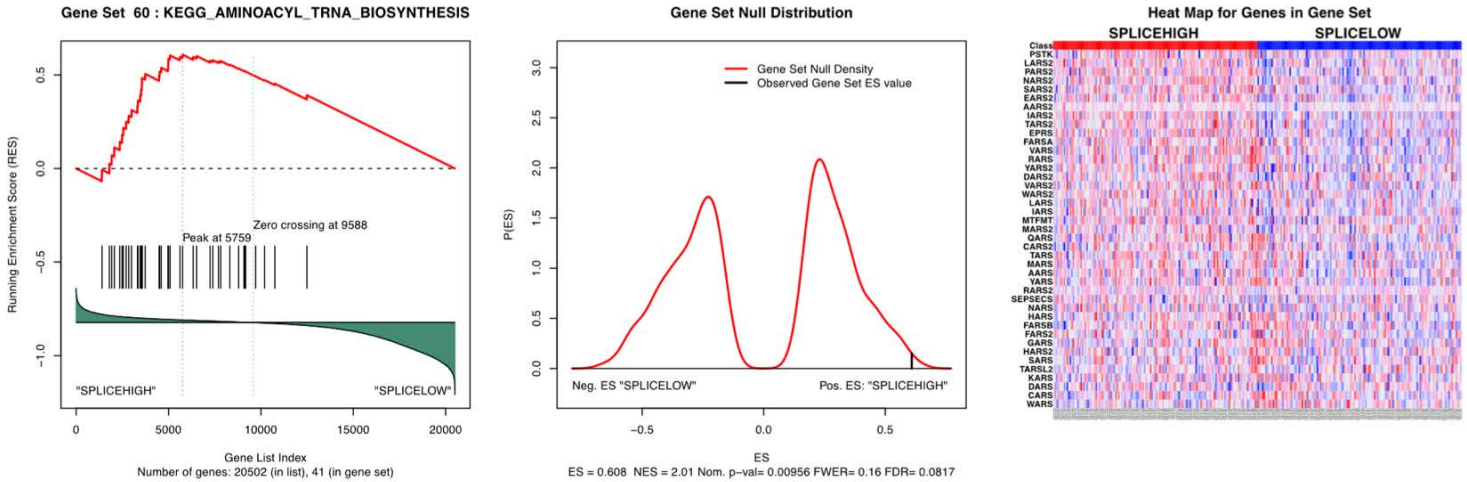


Selected pathways associated for **alpha** including gene set heatmap

Mitochondrial tRNA aminoacylation

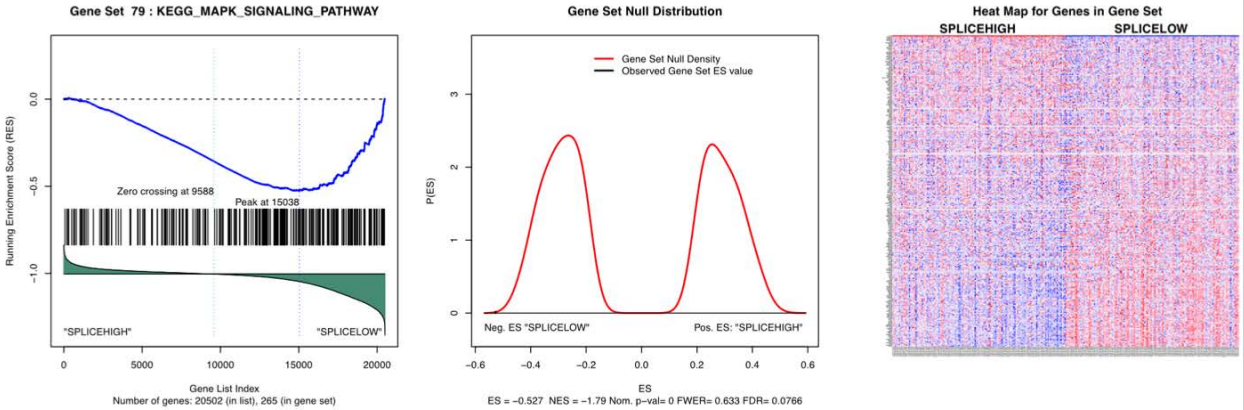


Aminoacyl tRNA biosynthesis

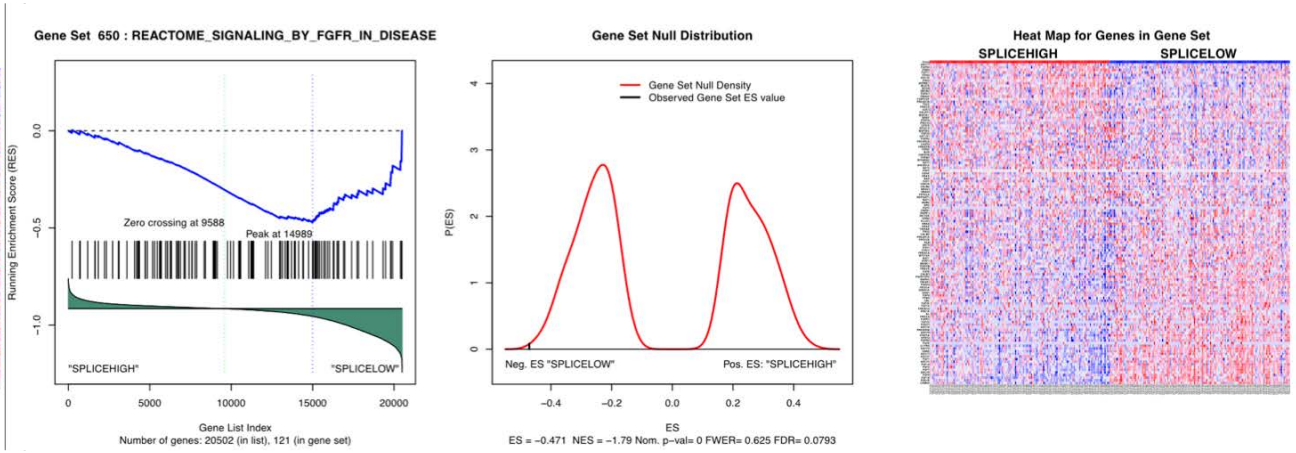


Selected pathways associated for **beta** including gene set heatmap

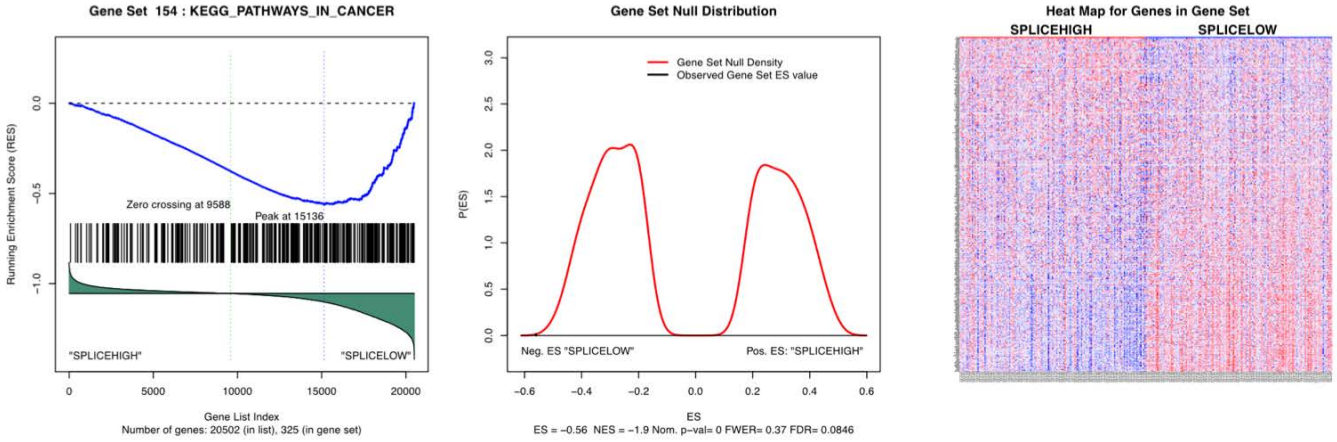
MAPK signaling



Signaling by FGFR in disease



Pathways in cancer



Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

a. Develop and use isoform specific antibodies

Mouse Monoclonal Antibody Development Using Hybridoma Technology

Antigen Analysis:

FGFR1 Alpha

MWSWKCLLFWAVLVTATLCTARPSPTLPEQAQPWGAPVEVESFLVHPGDLLQLRCRLRDD
VQSINWLRDGVQLAESNRTRITGEEVEVQDSVPADSGLYACVTSSPSGSDTTYFSVNVSD
ALPSSEDDDDDDSSSEEKETDNTKPNRMPVAPYWTSPEKMEKKLHAVPAAKTVKFKCPS
SGTPNPTLRWLKNGKEFKPDHRIGGYKVRYATWSIIMDSVVPSDKGNYTCIVENEYGSIN
HTYQLDVVERSPHRPILQAGLPANKTVALGSNVEFMCKVYSDPQPHIQWLKHIEVNGSKI
GPDNLPYVQILKTAGVNTTDKEMEVLHLRNVSFEDAGEYTCLAGNSIGLSHHSAWLTVLE
ALEERPAVMTSPLYLEIIIYCTGAFLISCMVGSVIVYKMKSGTKKSDFHSQMAVHKLAKS
IPLRRQVTVSADSSASMNSGVLLVRPSRLSSSGTPMLAGVSEYELPEDPRWELPRDRLVL
GKPLGEGCFGQVVLAEAIGLDKDKPNRVTKVAVKMLKSDATEKDLSDLISEMEMMKMIGK
HKNIINLLGACTQDGPLYVIVEYASKGNLREYLQARRPPGLEYCYNPSHNPEEQLSSKDL
VSCAYQVARGMEYLASKKCIHRDLAARNVLVTEDNVMKIADFGLARDIHHIDYYKKTTNG
RLPVKWMAPEALFDRIYTHQSDVWSFGVLLWEIFTLGGSPYPGVPVEELFKLLKEGHRMD
KPSNCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRIVALTSNQEYLDLSMPLDQYSPSF
PDTRSSTCSSGEDSVFSHEPLPEEPCLPRHPAQLANGGLKRR

FGFR1 Beta

MWSWKCLLFWAVLVTATLCTARPSPTLPEQDALPSSEDDDDDDDSSSEEKETDNTKPNRM
PVAPYWTSPEKMEKKLHAVPAAKTVKFKCPSSGTPNPTLRWLKNGKEFKPDHRIGGYKVR
YATWSIIMDSVVPSDKGNYTCIVENEYGSINHTYQLDVVERSPHRPILQAGLPANKTVAL
GSNVEFMCKVYSDPQPHIQWLKHIEVNGSKIGPDNLPYVQILKTAGVNTTDKEMEVLHLR
NVSFEDAGEYTCLAGNSIGLSHHSAWLTVLEALEERPAVMTSPLYLEIIIYCTGAFLISC
MVGSVIVYKMKSGTKKSDFHSQMAVHKLAKSIPLRRQVTVSADSSASMNSGVLLVRPSRL
SSSGTPMLAGVSEYELPEDPRWELPRDRLVLGKPLGEGCFGQVVLAEAIGLDKDKPNRVT
KVAVKMLKSDATEKDLSDLISEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYASKGNL
REYLQARRPPGLEYCYNPSHNPEEQLSSKDLVSCAYQVARGMEYLASKKCIHRDLAARNV
LVTEDNVMKIADFGLARDIHHIDYYKKTTNGRLPVKWMAPEALFDRIYTHQSDVWSFGVL
LWEIFTLGGSPYPGVPVEELFKLLKEGHRMDKPSNCTNELYMMMRDCWHAVPSQRPTFKQ
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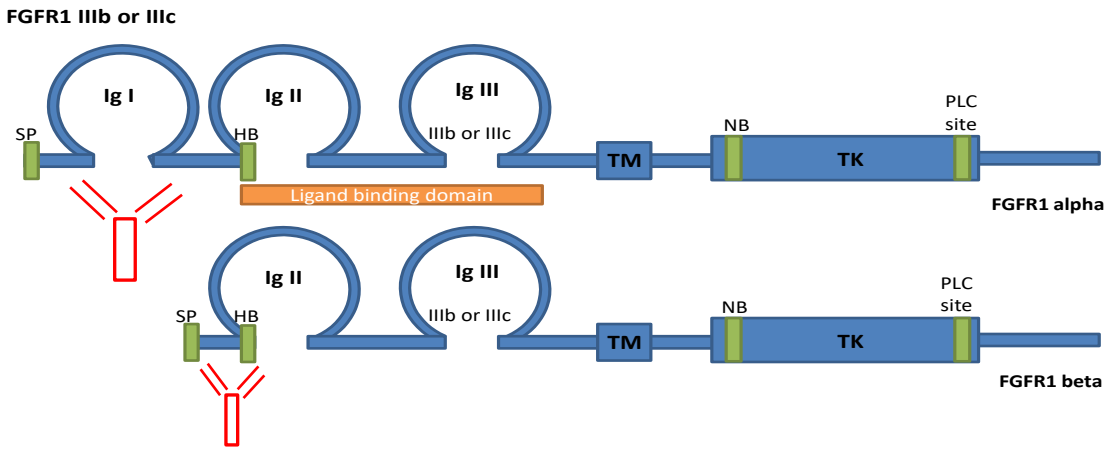
HPAQLANGGLKRR



Design of peptides used as immunogens to develop the antibodies

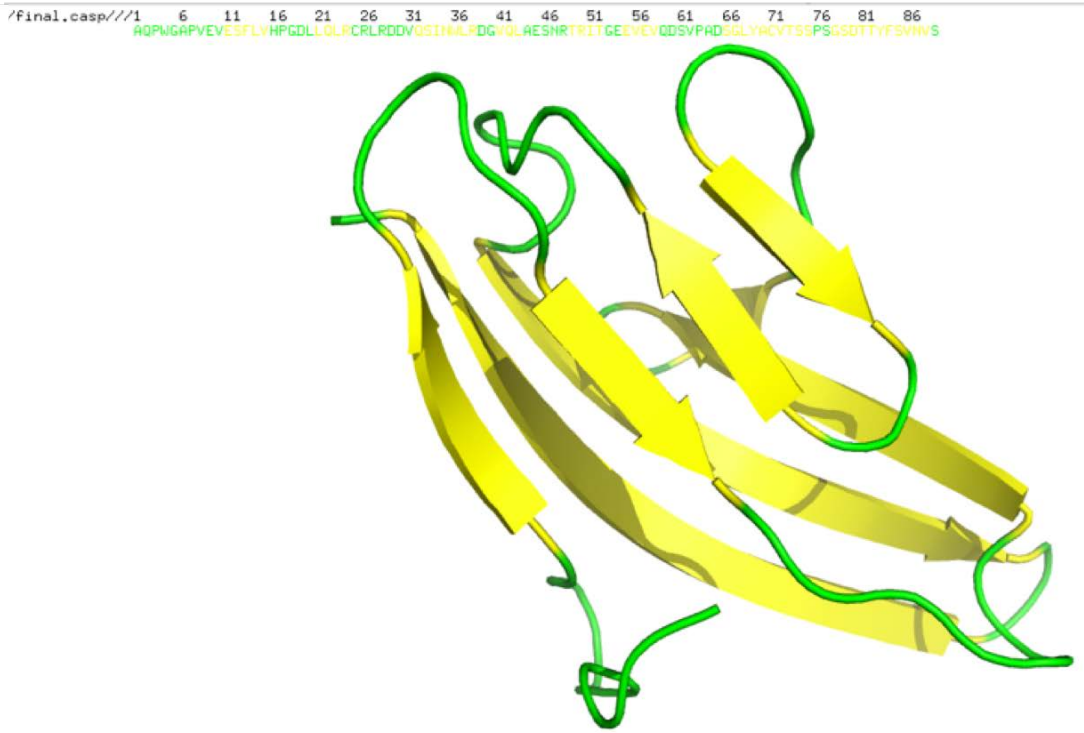
Customized Antibodies Peptide Design

Sequence blast results

Score	Expect	Method	Identities	Positives	Gaps
97.4 bits(241)	5e-25	Compositional matrix adjust.	87/309(28%)	123/309(39%)	86/309(27%)

```
Query  1    MWSWKCLLFWAVLVTATLCTARPSPTLPEQ------------------------------  30
            MWSWKCLLFWAVLVTATLCTARPSPTLPEQ
Sbjct  1    MWSWKCLLFWAVLVTATLCTARPSPTLPEQDALPSSEDDDDDDDSSSEEKETDNTKPNRM  60

Query  31   --AQPWGAPVEVESFL--VHPGDLLQLRCRLRDDVQ-SINWLRDGVQLAESNRTRITGEE  85
               A  W +P ++E   L  V      ++ +C         ++ WL++G +     +  RI G +
Sbjct  61   PVAPYWTSPEKMEKKLHAVPAAKTVKFKCPSSGTPNPTLRWLKNGKEFKPDH--RIGGYK  118

Query  86   VE------VQDS-VPADSGLYACVTSSPSGSDTTYFSVNVSDALPSSEDDDDDDDSSSEE  138
            V       + DS VP+D G Y C+  +  GS     + ++V +
Sbjct  119  VRYATWSIIMDSVVPSDKGNYTCIVENEYGSINHTYQLDVVE-----------------  160

Query  139  KETDNTKPNRMPVAPYWTSPEKMEKKLHAVPAAKTVKFKCPSSGTPNPTLRWLK----NG  194
                      R P   P    +      K     V     V+F C      P P ++WLK    NG
Sbjct  161  ---------RSPHRPILQAGLPANK---TVALGSNVEFMCKVYSDPQPHIQWLKHIEVNG  208

Query  195  KEFKPDH-------RIGGYKVRYATWSII-MDSVVPSDKGNYTCIVENEYGSINHTYQLD  246
              +  PD+       +  G          ++ + +V   D G YTC+  N  G  +H+  L
Sbjct  209  SKIGPDNLPYVQILKTAGVNTTDKEMEVLHLRNVSFEDAGEYTCLAGNSIGLSHHSAWLT  268

Query  247  VVERSPHRP  255
            V+E    RP
Sbjct  269  VLEALEERP  277
```

3D structure of the EXTRA Ig-like domain of FGFR1 alpha



Peptide1 (alpha)- aa 31 to 59:
AQPWGAPVEVESFLVHPGDLLQLRCRLRDDVQSINWLRDGVQLAESNRTRITG
EEVEVQDSVPADSGLYACVTSSPSGSDTTYFSVNVS
Peptide2 (beta)- aa 21 to 41:
ARPSPTLPEQDALPSSEDDD

Customized Antibodies

Clones for test

Project ID	Target protein	Product Name	The Epitope Identification/Peptide sequence	Product type	Powder or Liqiud	Weight (mg)	Elisa Titer (K)/ Detection limit (ng)*
28090-1	FGFR1 Alpha	28090-1-1/2M16-B	PGDLLQLRCRLRDD	Ascites	powder	0.2	5ng
28090-1	FGFR1 Alpha	28090-1-4/C1	PGDLLQLRCRLRDD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-4/C2	PGDLLQLRCRLRDD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-4/C3	PGDLLQLRCRLRDD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-5/C4	LRDGVQLAESNRTR	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-5/C5	LRDGVQLAESNRTR	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-5/C6	LRDGVQLAESNRTR	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-6/C7	NRTRITGEEVEVQD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-6/C8	NRTRITGEEVEVQD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	28090-1-6/C9	NRTRITGEEVEVQD	Ascites	powder	0.2	0.25ng
28090-1	FGFR1 Alpha	D28090-1-1	PGDLLQLRCRLRDD	peptide	powder	3	
28090-1	FGFR1 Alpha	D28090-1-2	LRDGVQLAESNRTR	peptide	powder	3	
28090-1	FGFR1 Alpha	D28090-1-3	NRTRITGEEVEVQD	peptide	powder	3	

Project ID	Target protein	Product Name	The Epitope Identification/Peptide sequence	Product type	Powder or Liqiud	Weight (mg)	Elisa Titer (K)/ Detection limit (ng)*
28089-1	FGFR1 Beta	28089-1-4/C1	RPSPTLPEQDALPS	Ascites	powder	0.2	0.05ng
28089-1	FGFR1 Beta	28089-1-4/C2	RPSPTLPEQDALPS	Ascites	powder	0.2	0.05ng
28089-1	FGFR1 Beta	28089-1-4/C3	RPSPTLPEQDALPS	Ascites	powder	0.2	0.25ng
28089-1	FGFR1 Beta	D28089-1-1	RPSPTLPEQDALPS	peptide	powder	1	

recist
CJ
1mg/ml
1mg/ml
1mg/ml

Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

a. Develop and use isoform specific antibodies

Test the specificity and selectivity of customized antibodies

Ascites (supernatants) provided for pre-screen: 12 for FGFR1 alpha
3 for FGFR1 beta

>3 ICC (different protocol conditions tested) → Beta antibody recognizing cells expressing alpha isoform

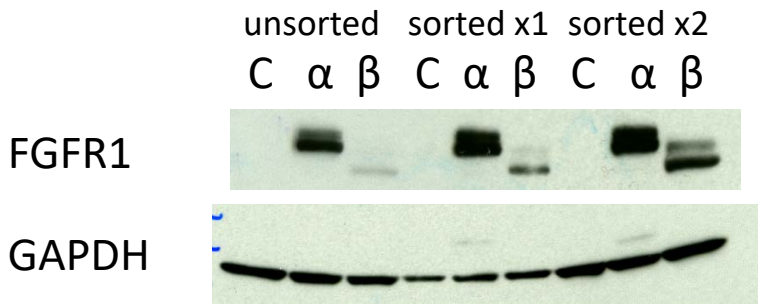
Wb → Unspecific bands

Final goal: IHC on formalin fixed paraffin embedded tissue samples

↓

optimization of cell preparation protocol for IHC of fixed paraffin embedded cell pellets

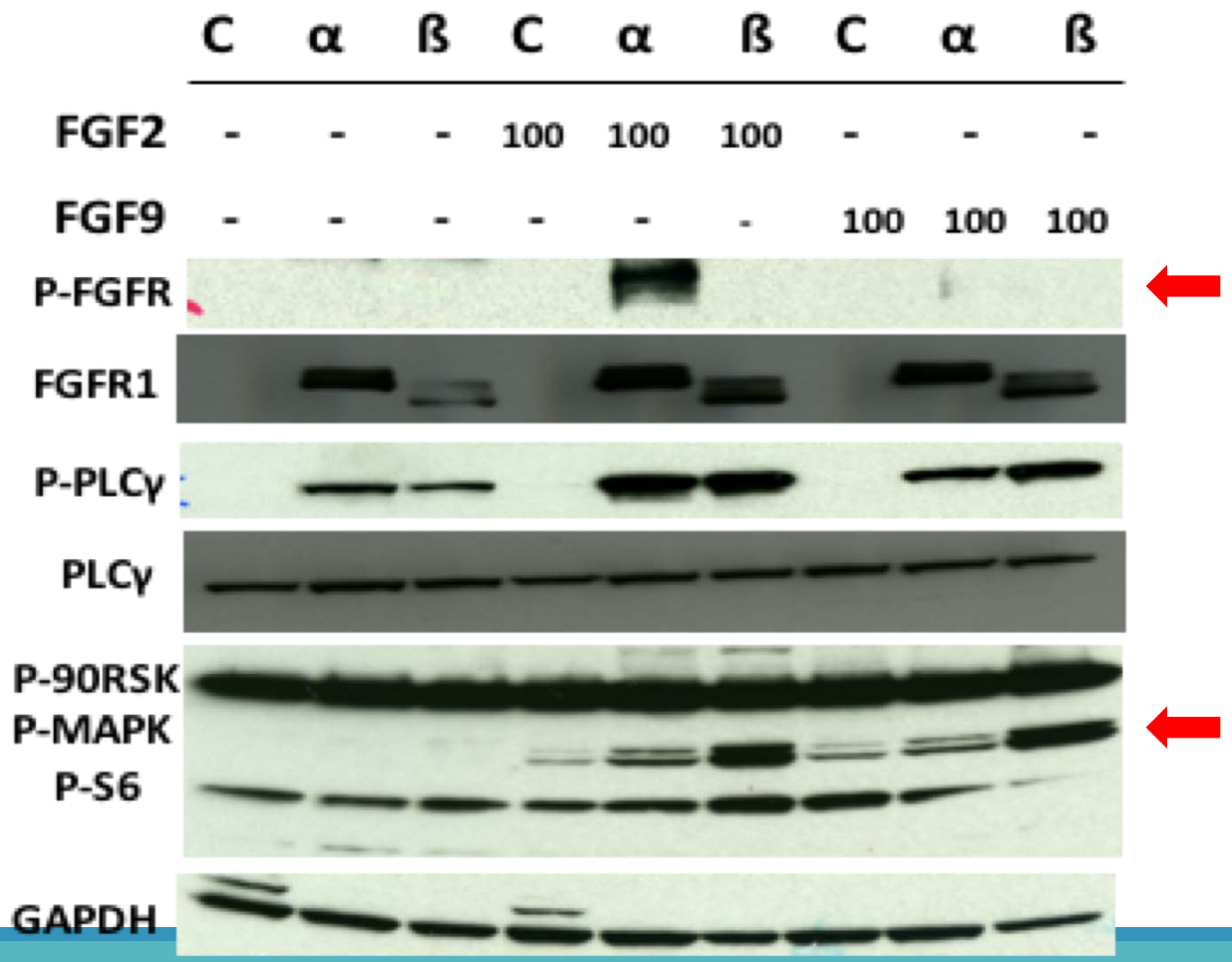
FGFR1 expression in **C42B** stable cell lines 03-16-17



Specific Aim 1. To study FGFR1 isoforms expression in human PCa and its molecular and clinical correlates

c. Study the signaling cascade induced by FGFR1 alpha and beta in PCa cells

C4-2B EV
C4-2B FGFR1 alpha → Serum-free media 2h → + HSPG 1h → + FGF2/FGF9 45' → Western blot
C4-2B FGFR1 beta

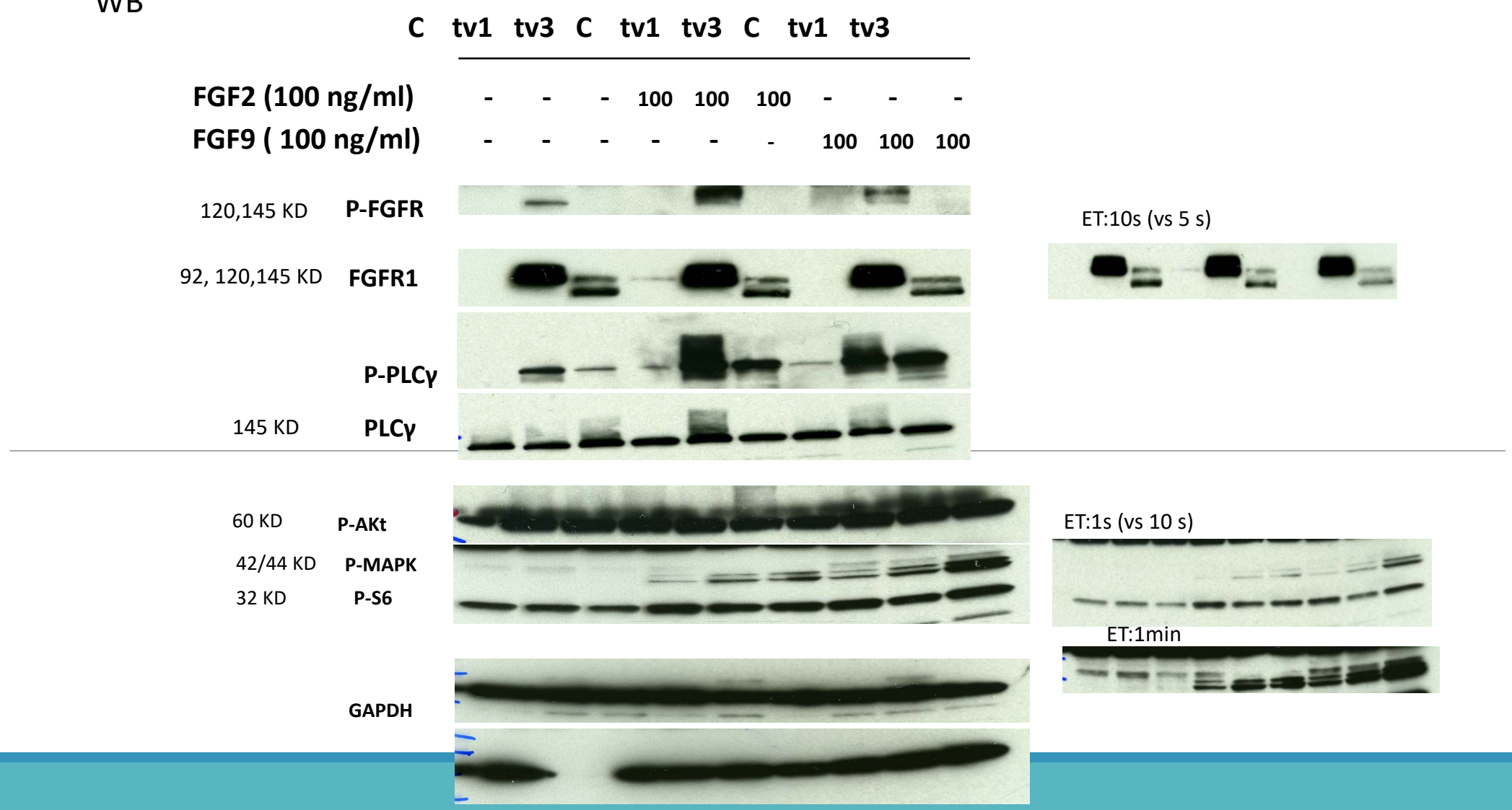


3h starve

C4-2B cells stables FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

May/ 5/2017

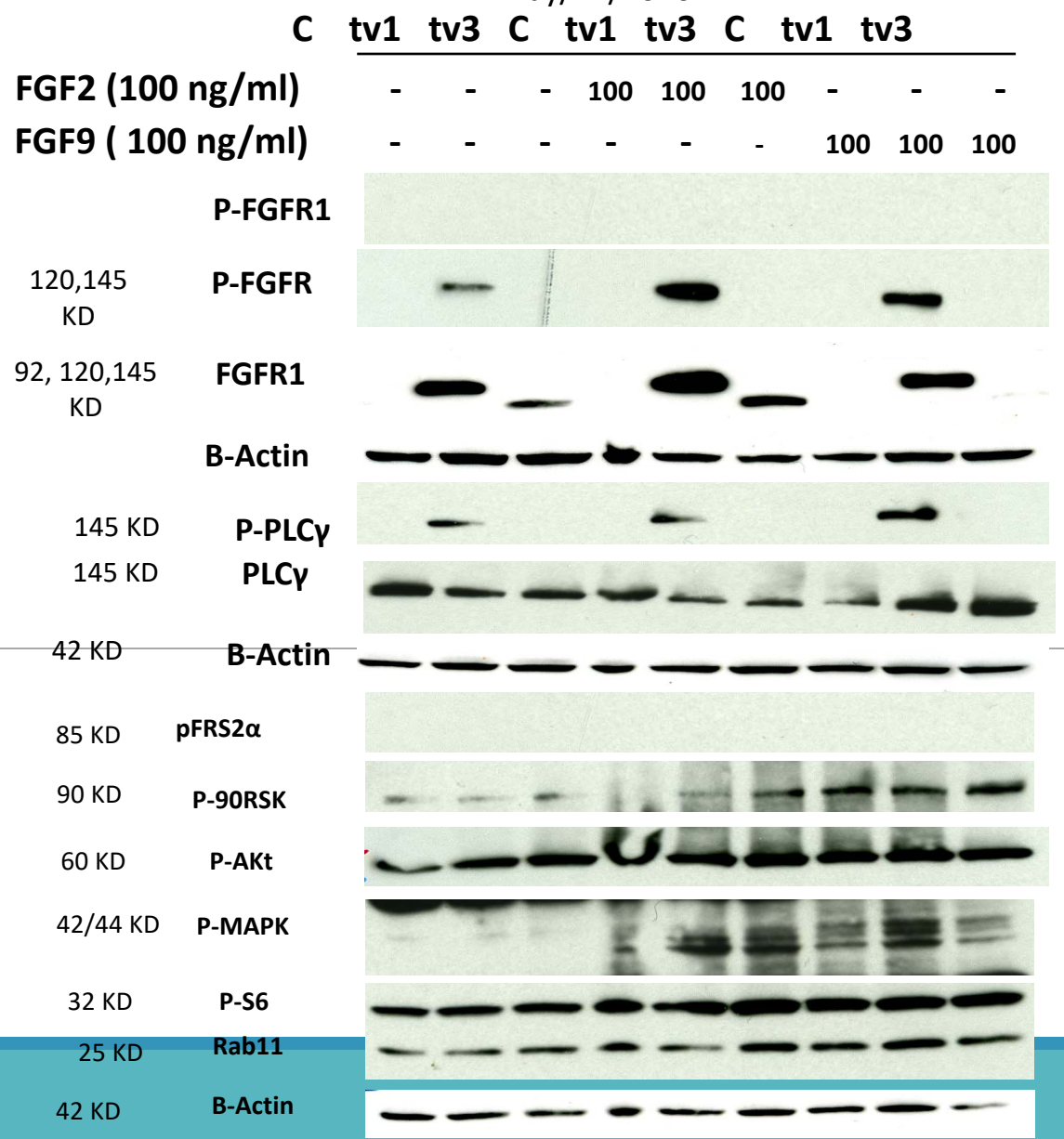
WB



Exp 1 C4-2B cells with transient expression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

WB

May/12/2016

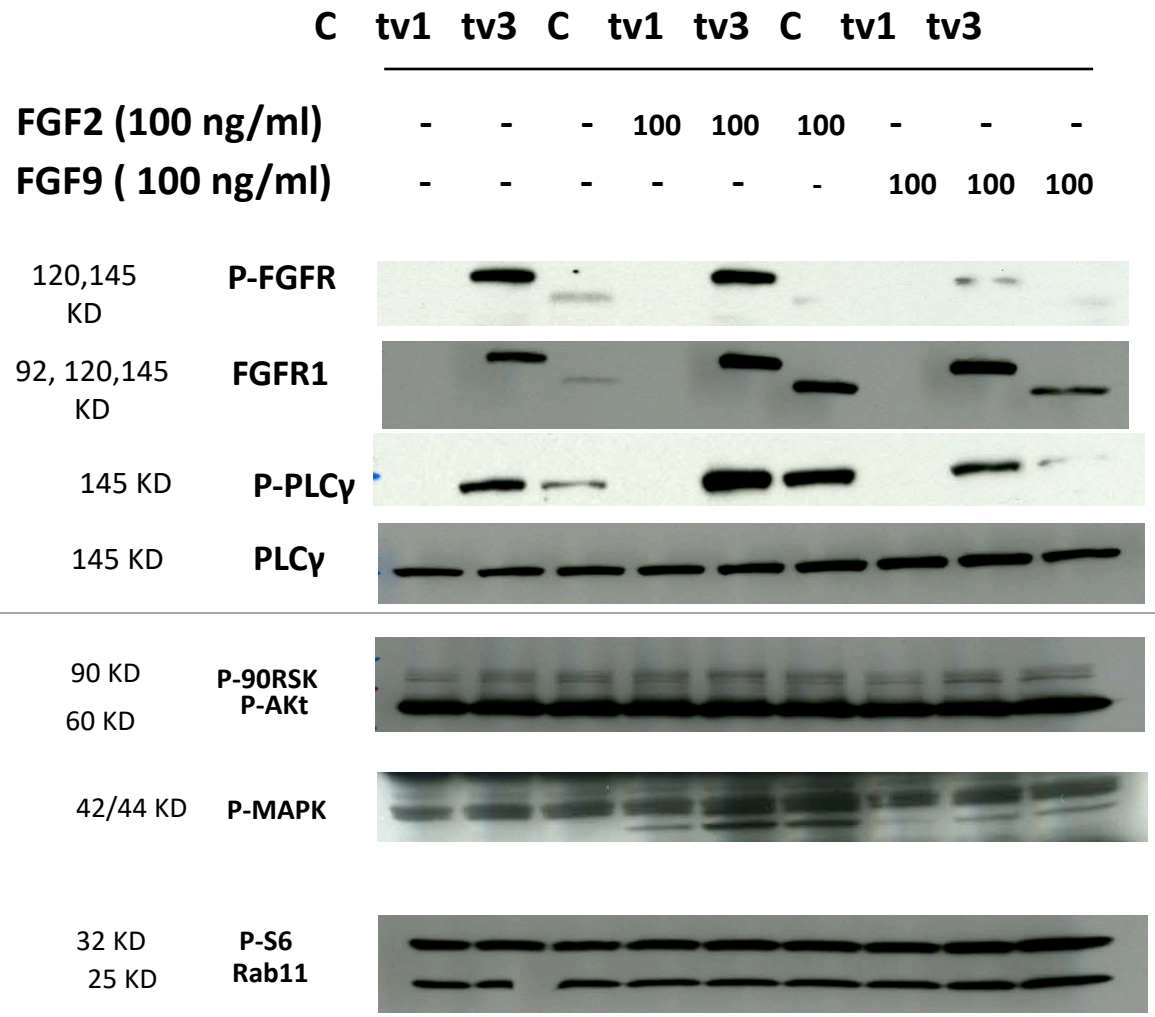


Exp 2

C4-2B cells with transient expression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

June/06/2016

WB



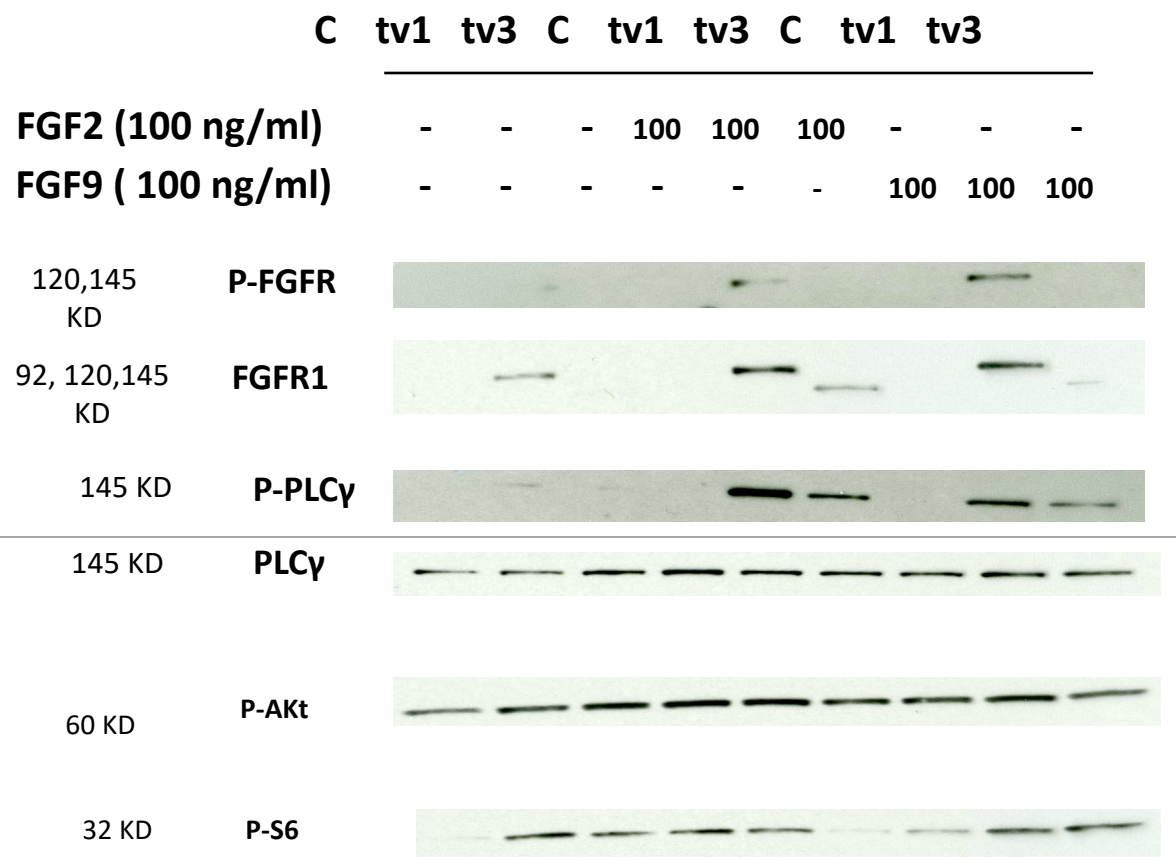
Exp 2- bis

C4-2B cells with transient expression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

June/21/2016

same samples as june 6, but diluted

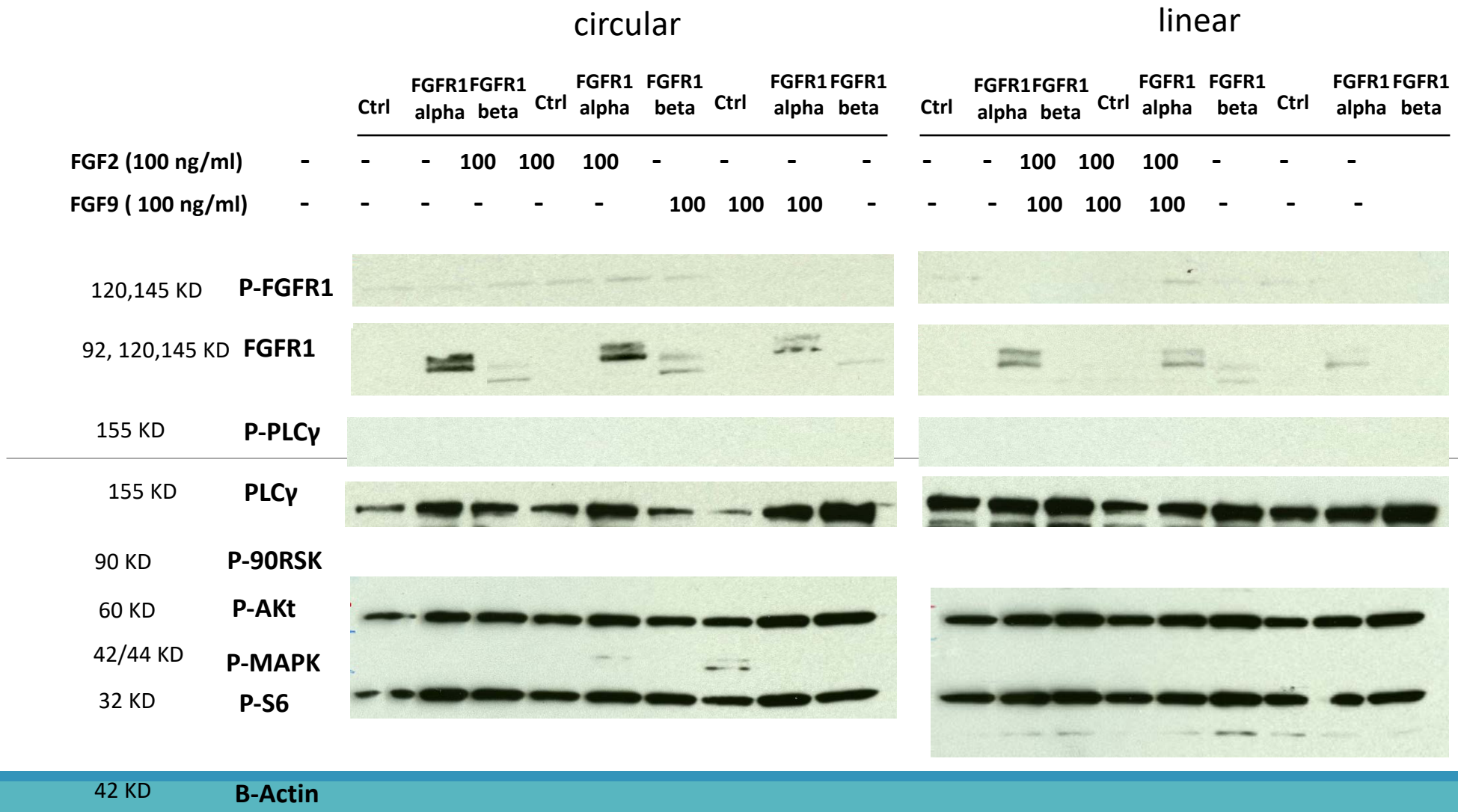
WB



“ Exp 1”

PC3 cells with stable overexpression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

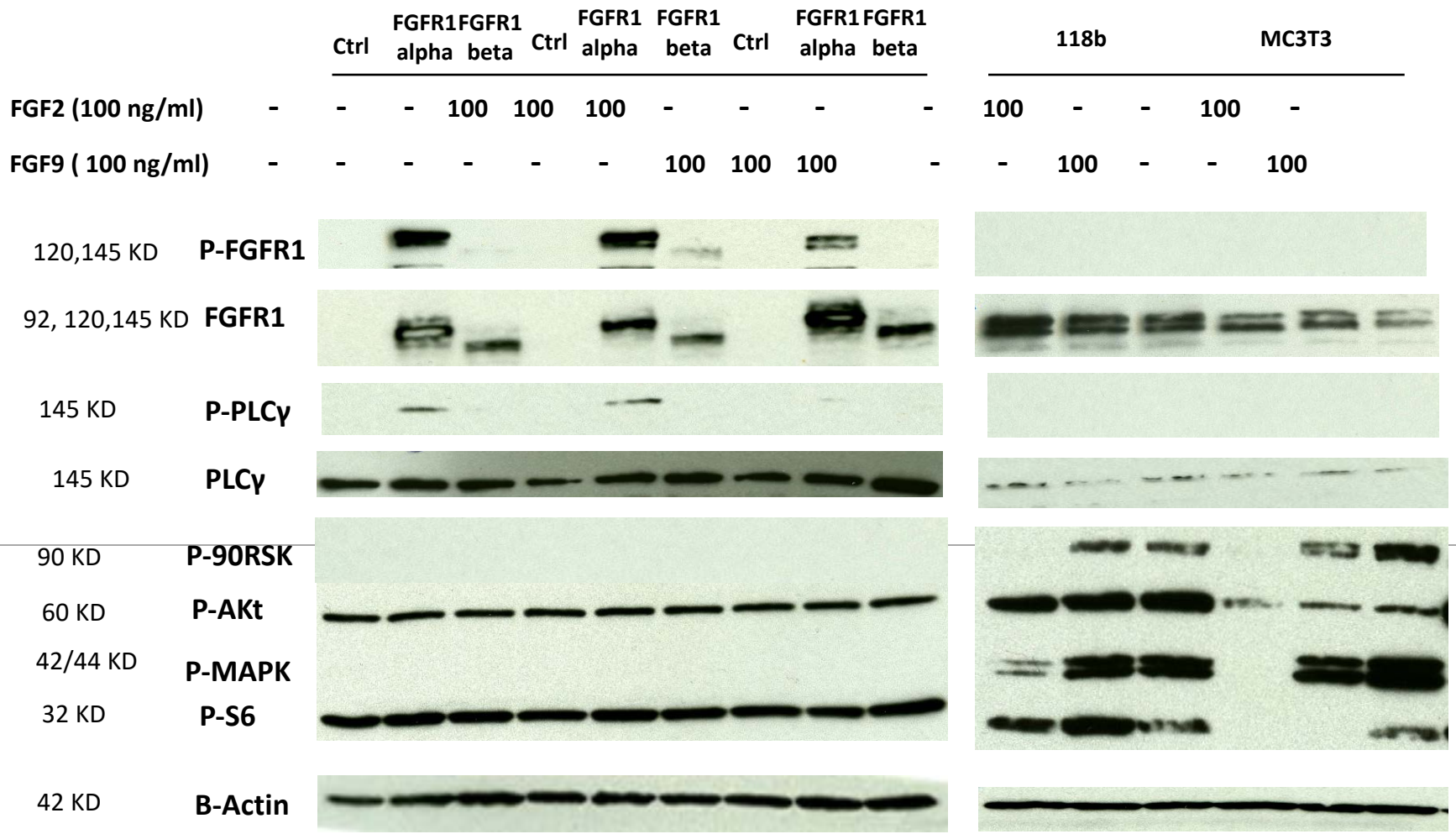
Apr/30/2015



Exp 1

PC3 cells with transient overexpression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

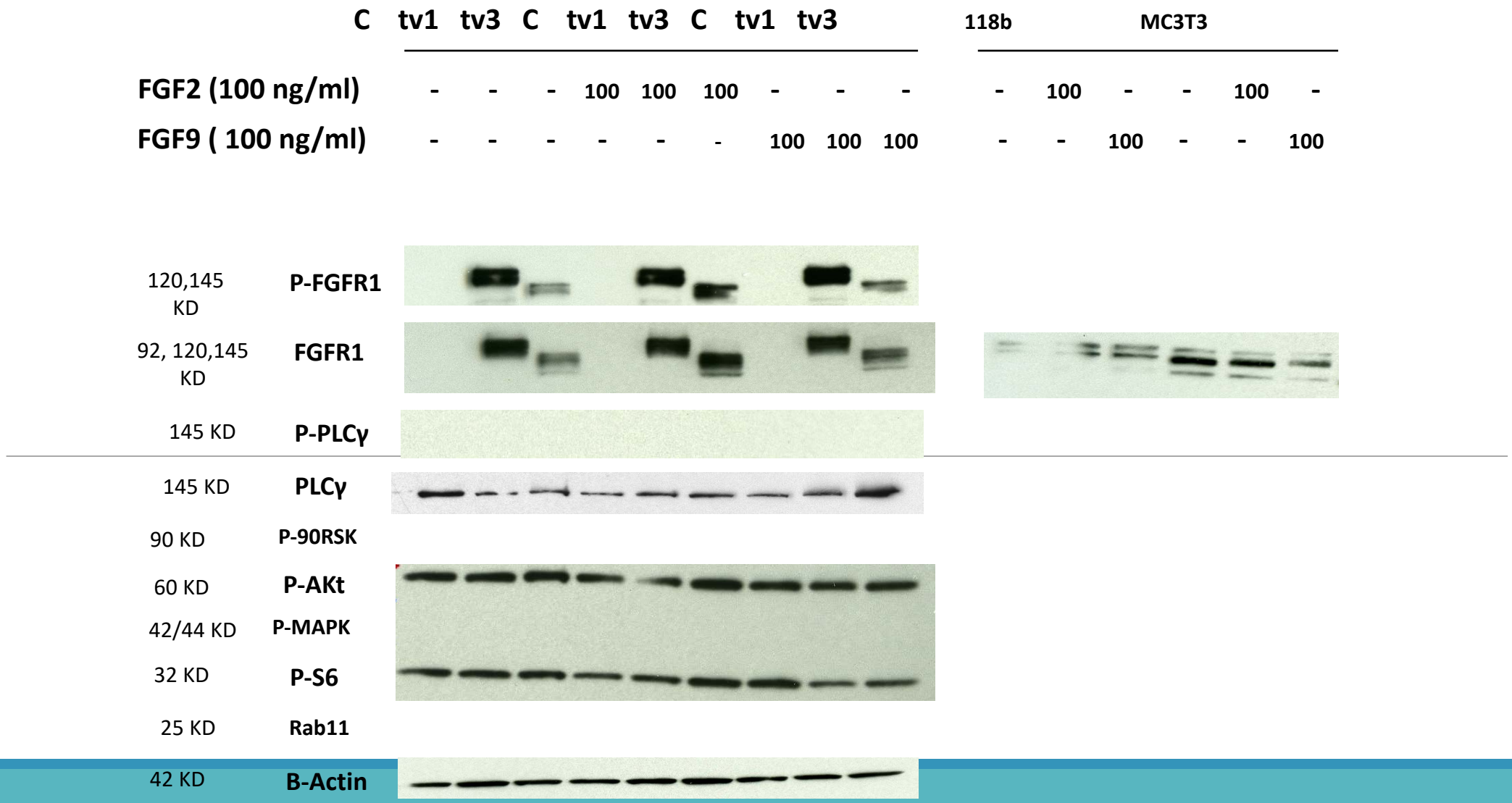
Mar/19/2015



Exp 2

PC3 cells with transient overexpression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

Sep/22/2015

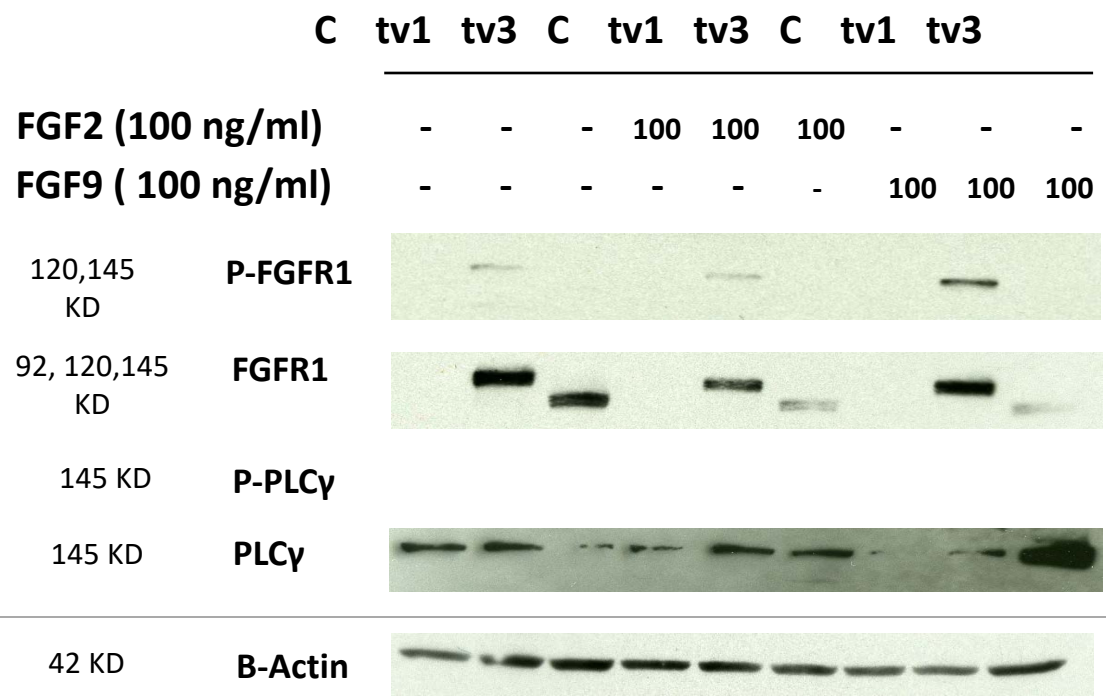


Exp 3

PC3 cells with transient overexpression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

Nov/24/2015

WB

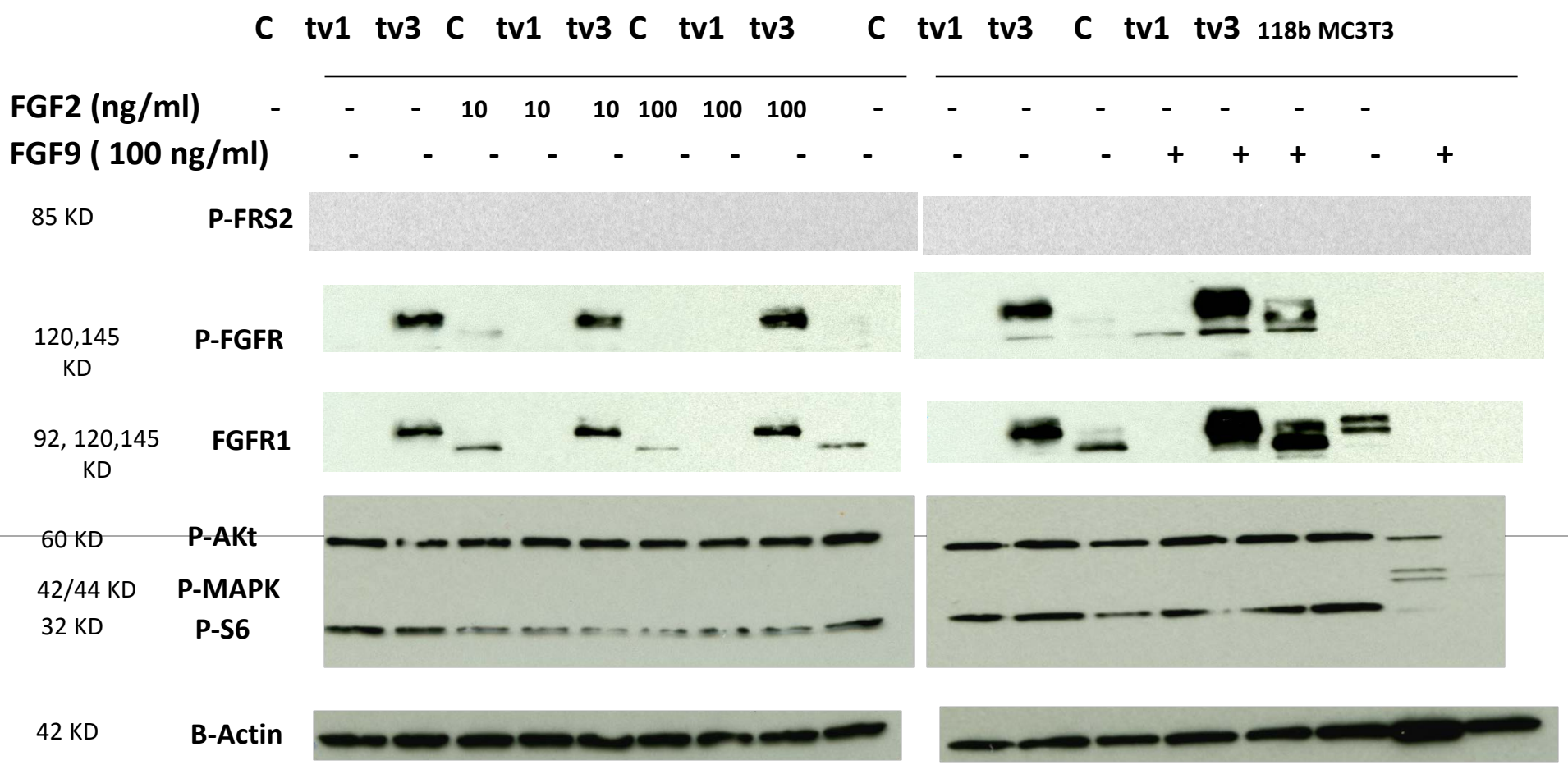


Exp 0

PC3 cells with transient overexpression of FGFR1 tv1 and tv3 isoforms treated with FGF2 and FGF9

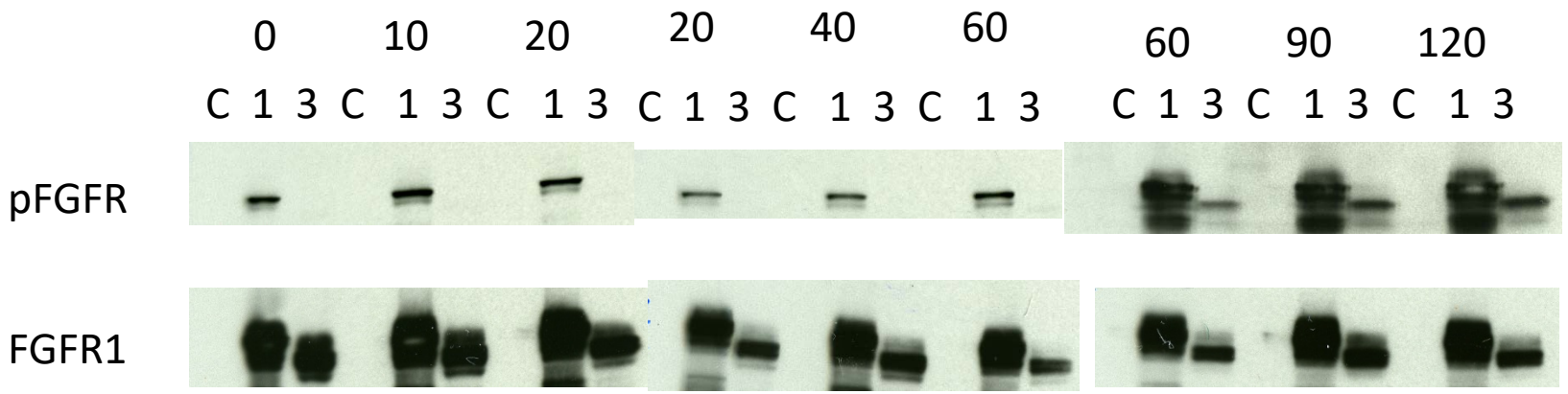
OJO! Different arrengment

Feb/05/2015



Without HSPG?

PFGFR1 AND TOTAL FGFR1 IN PC3 CELLS INDUCED WITH FGF2 LIGAND AT DIFFERENT TIME-POINTS (SHORT)

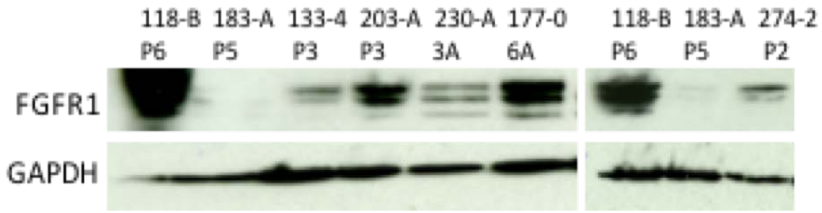


Used femto ECL for pFGFR

ET: 1 sec

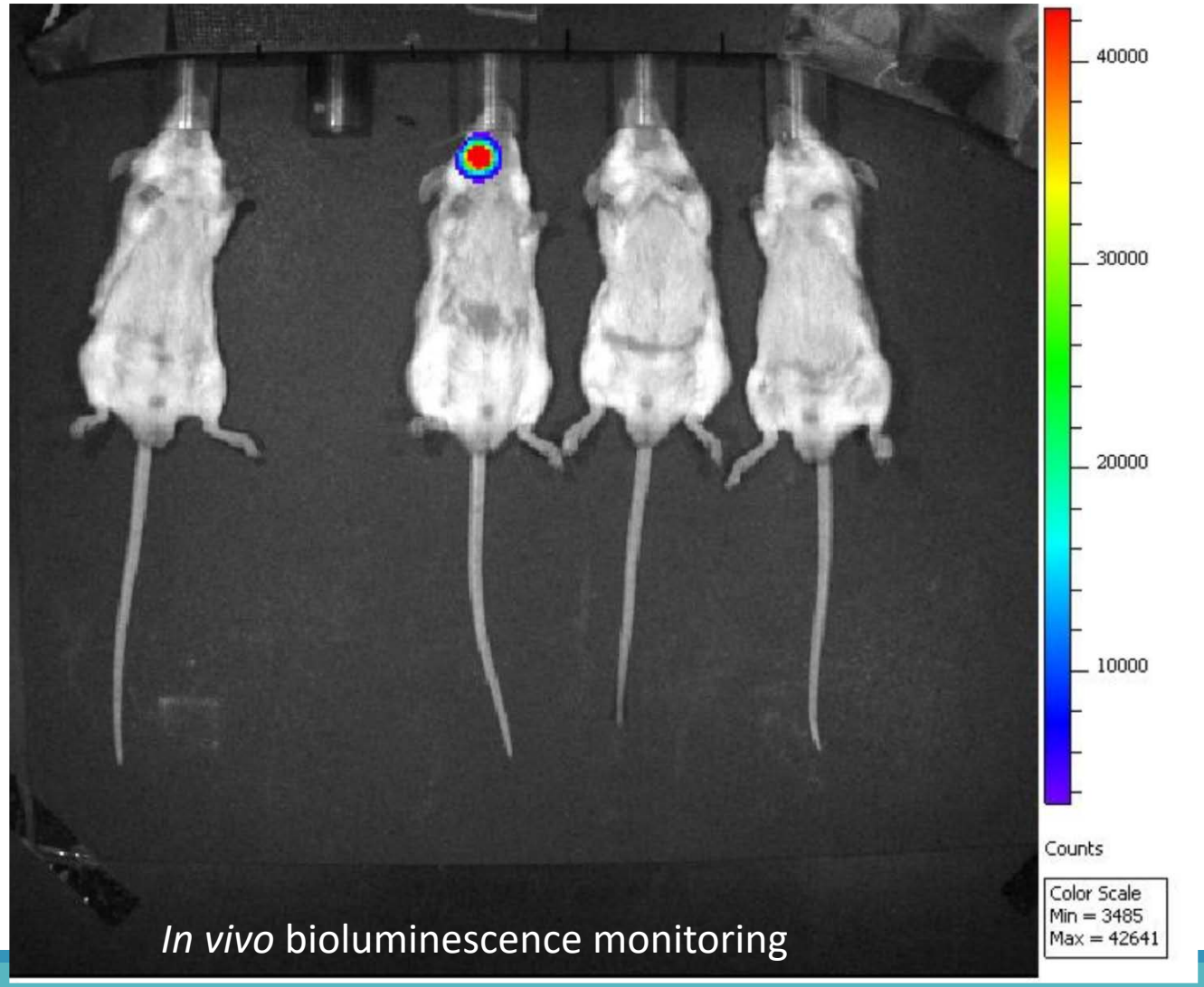
Expression of FGFR1 in PDXs

Wb



Specific Aim 2. To assess the role of FGFR1 (and its isoforms) in the growth of PCa in bone and PCa bone interaction

a. Evaluate the metastatic dissemination of PCa cells driven by FGFR1 isoforms



In vivo bioluminescence monitoring

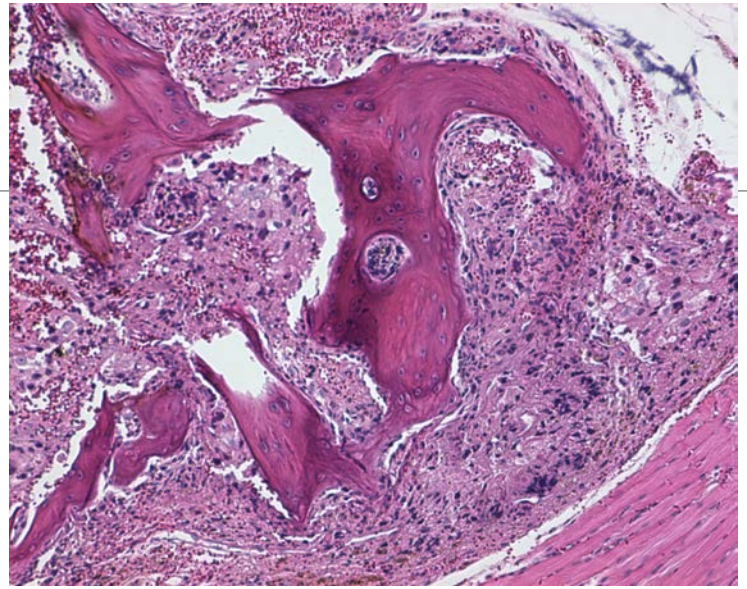
luciferase

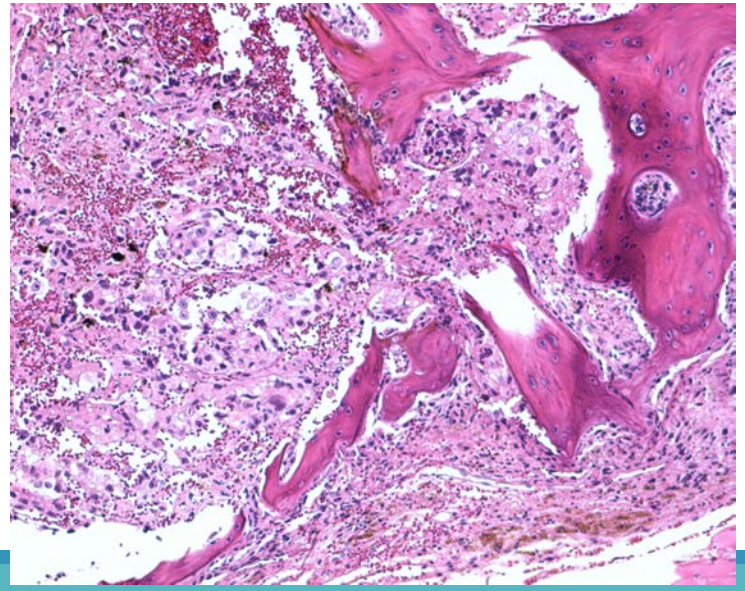
C4-2B EV

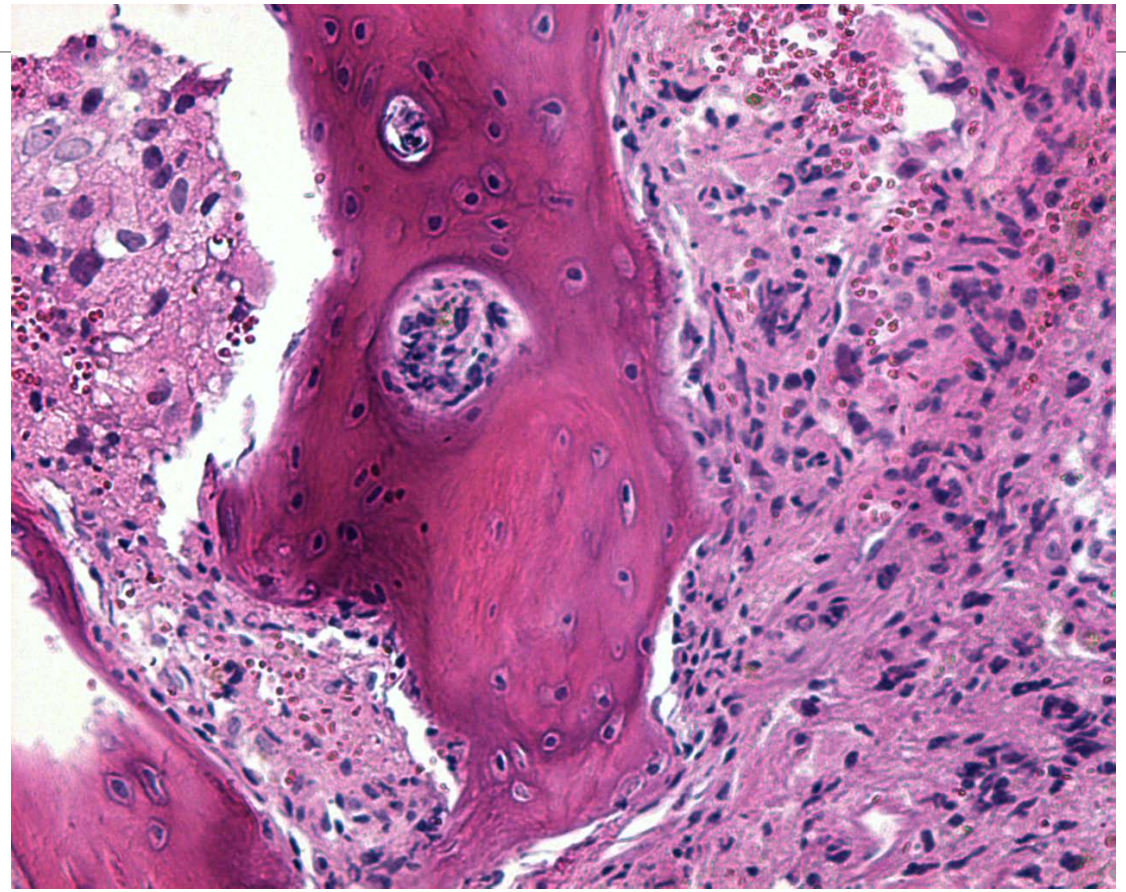
C4-2B FGFR1 alpha

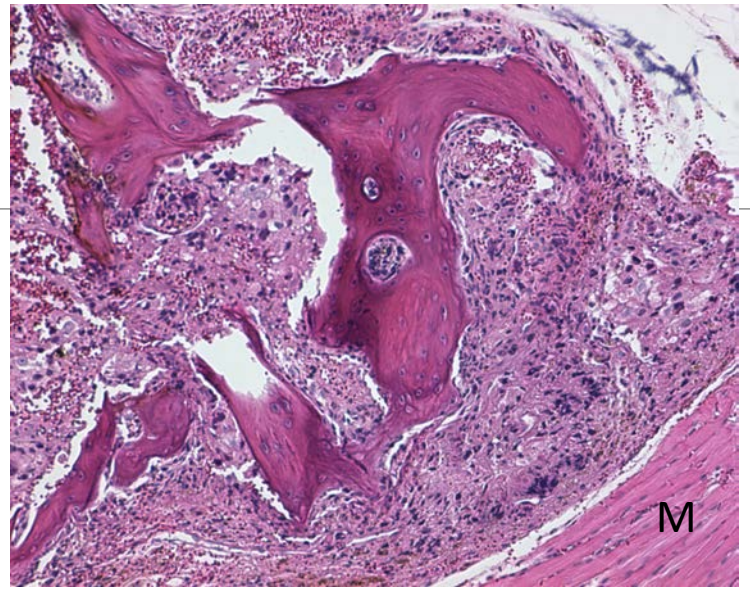
C4-2B FGFR1 beta

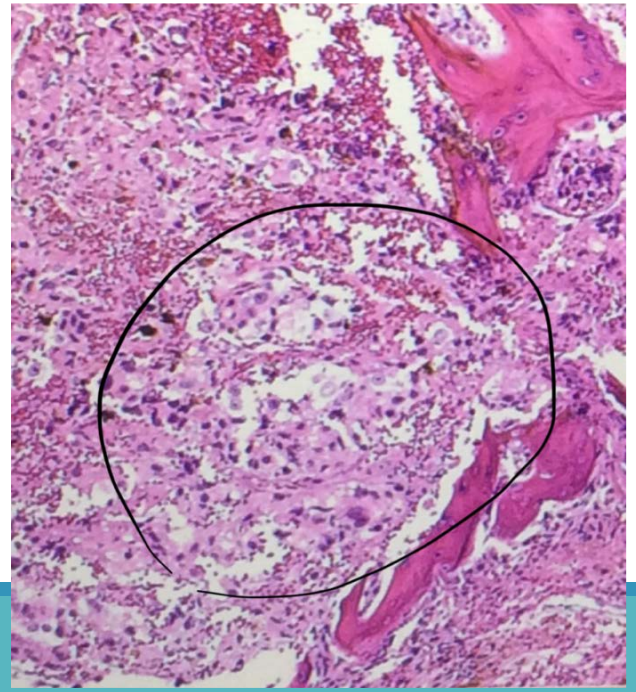
HE right mandible mouse #2

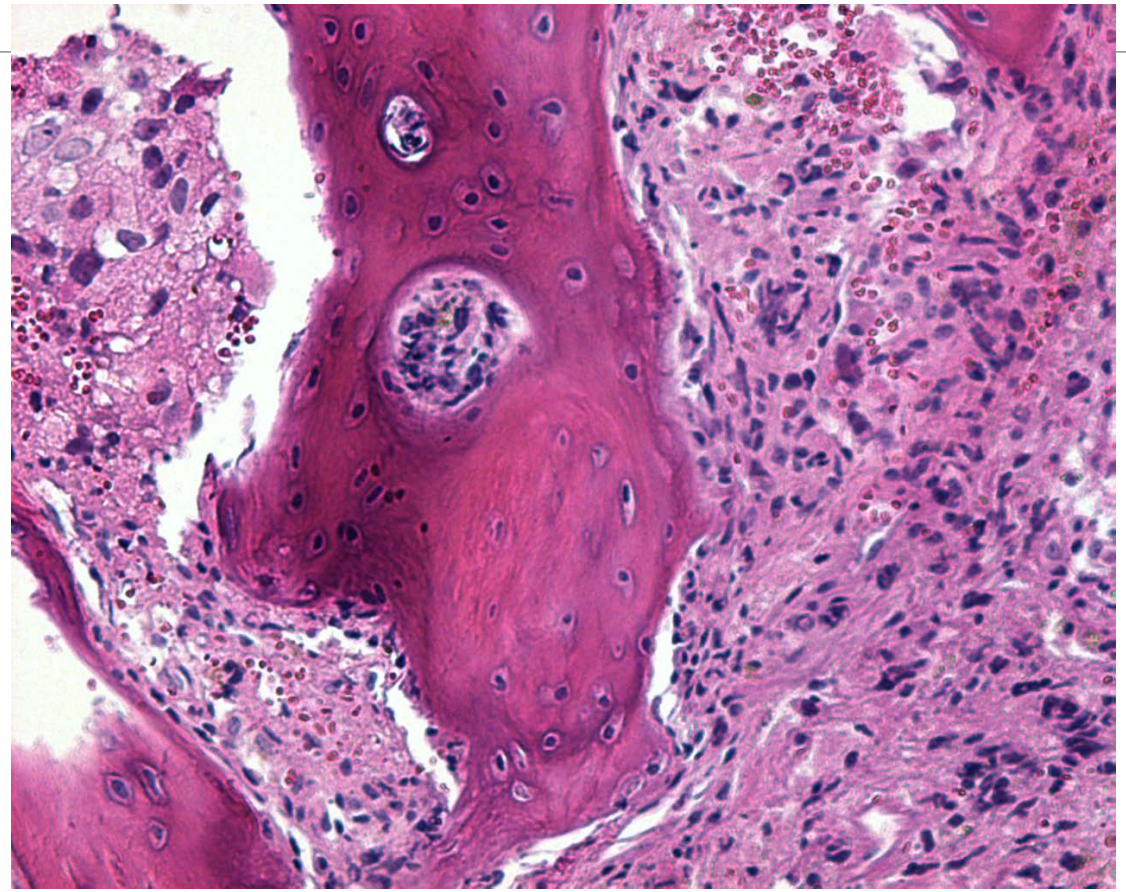







M





FGFR inhibitors

IC50 (nM)	FGFR1	FGFR2	FGFR3	FGFR4	Activity against VEGF	
AZD4547 (AstraZeneca)	0.2	1.8	2.5	165	Yes	
BGJ398 (Novartis)	0.9	1.4	1	60	No	
JNJ-42756493 (J&J, Janssen Pharmaceutical Companies)	<1	<1	1.05	<1	No	
Dovitinib (Novartis)	8	40	9		Yes	

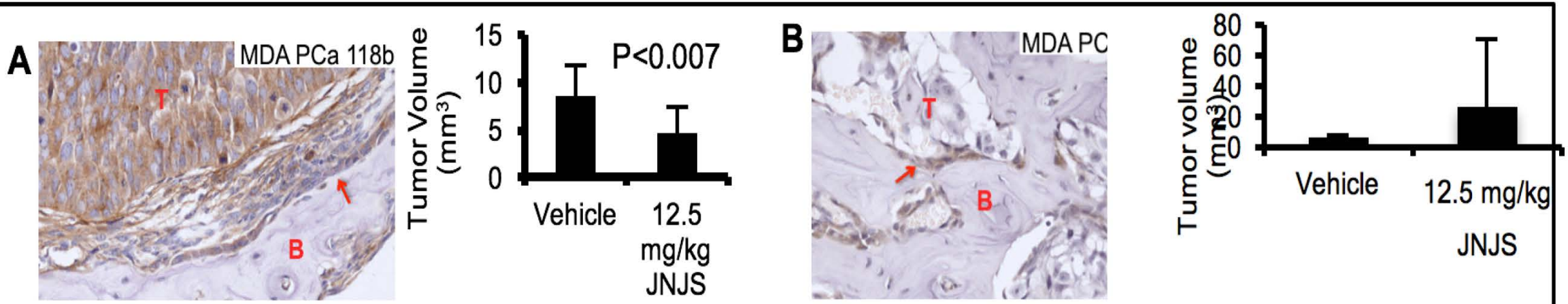
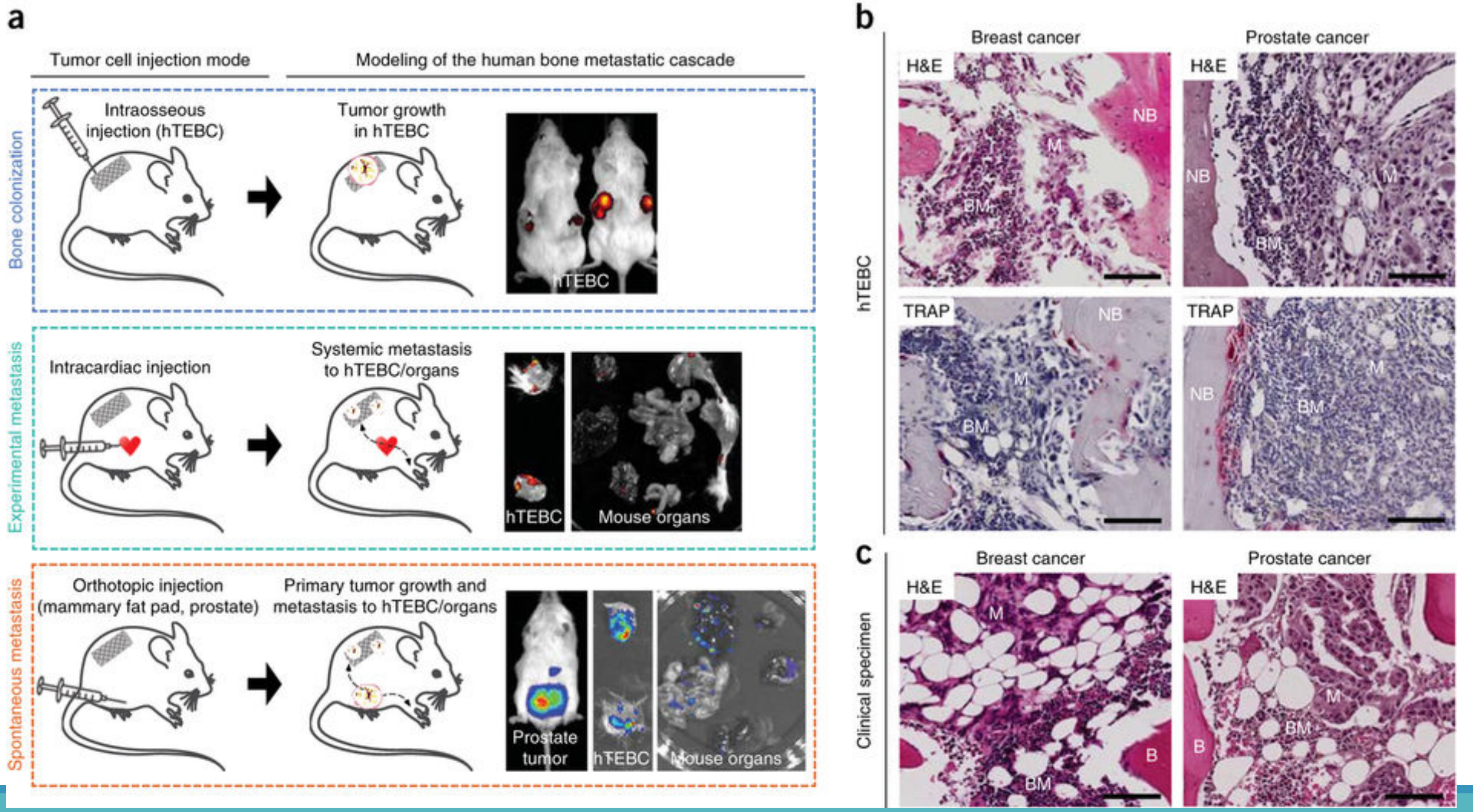


Fig 2. Immunohistochemical analysis of FGFR1 expression in MDA PCa 118b (**A**-left panel) and MDA PCa 183 (**B**-left panel) PDXs growing subcutaneously in SCID mice. Tumor volume measured from serial sagittal MR images of femurs bearing MDA PCa 118b (**A**-right panel) and MDA PCa 183 (**B**-right panel) derived tumors in control and treated mice. T, tumor; B, bone; Arrow, osteoblasts


a
Tumor cell injection mode
Modeling of the human bone metastatic cascade
Bone colonization
Intraosseous injection (hTEBC)
Tumor growth in hTEBC
hTEBC
Experimental metastasis
Intracardiac injection
Systemic metastasis to hTEBC/organs
hTEBC
Mouse organs
Spontaneous metastasis
Orthotopic injection (mammary fat pad, prostate)
Primary tumor growth and metastasis to hTEBC/organs
Prostate tumor
hTEBC
Mouse organs
b
hTEBC
Breast cancer
Prostate cancer
H&E
NB
M
BM
TRAP
c
Clinical specimen
B