The difficult targets: FGFR1/2

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- Employment or leadership Position: None
- **Advisory Role: Ad Hoc Advisory Boards/Consultations (most recent contact last 3 years):**
  - 2016: G1 Therapeutics, Orion, Clovis
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  - 2014: Eli Lilly, Immunogen, Novartis, Genentech/Roche, Biodesix, AstraZeneca
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- Other Remuneration: None

FGFR1 as a targetable driver oncogene in lung cancer

- Problems with the predictive markers in lung cancer
- Problems with the drugs

FGFR2 as a targetable driver oncogene in lung cancer

- Same drug issues
- Rarity of abnormalities

FGF family

- 22 FGFs, of which 18 considered biologically active, 6 subfamilies
  - Secreted
  - Heparin sulfate proteoglycan bound (stabilization, protease protection)
    - *Endocrine Factors*
      - FGF19, 21 and 23
      - Involved in bile acid, cholesterol, glucose, vit D and phosphate homeostasis
      - Dependent on klotho proteins (klotho or β–klotho) in target tissue
    - *Paracrine/Autocrine Factors*

Dientsmann et al, Annals Onc 2013
Kelleher et al, Carcinogenesis 2013

FGFR family

- 4 cell surface receptors FGFR1-4
- Ligand specificity affected by splice variants of FGFR1-3 (may limit autocrine effects in normal physiology)

Dientsmann et al, Annals Onc 2013
Kelleher et al, Carcinogenesis 2013

Normal physiology (other than endocrine)

- Angiogenesis (VEGFR, PDGFR interaction)
- Musculoskeletal development

Table II. Germline and somatic genetic alterations of FGFR gene receptors

Gene	Genetic alteration	Syndrome
FGFR1	P252R mutation	Pfeiffer syndrome (46)
	G48S and L245P mutations	Idiopathic hypo-gonadotropic hypo-gonadism (48)
	Heterozygous loss of function mutations	Kallmann syndrome type 2 (10%) (50)
	Y372C mutation	Osteoglophonic dysplasia (52)
FGFR2	C342R mutation	Jackson-Weiss syndrome (53)
	W290G and C342W mutations	Crouzon syndrome (53)
	S252W and P253R mutations	Apert syndrome (56)
	W290C, Y340C mutations	Pfeiffer syndrome (58)
	Y375C	Beare-Stevenson cutis gyrata syndrome (60)
	K526E mutation	Familial scaphocephaly syndrome (62)
	I156R LADD mutation	Lacrimo-auriculo-dento-digital syndrome (64)
FGFR3	G1138A	Achondroplasia (66)
	Ala391Glu substitution	Crouzon syndrome with acanthosis nigricans (68)
	C742T mutation	Thanatophoric dysplasia type I and type II (33)
	Asn540Lys substitution	Hypochondroplasia (71)
FGFR4	—	—

Pfeiffer syndrome

Achondroplasia

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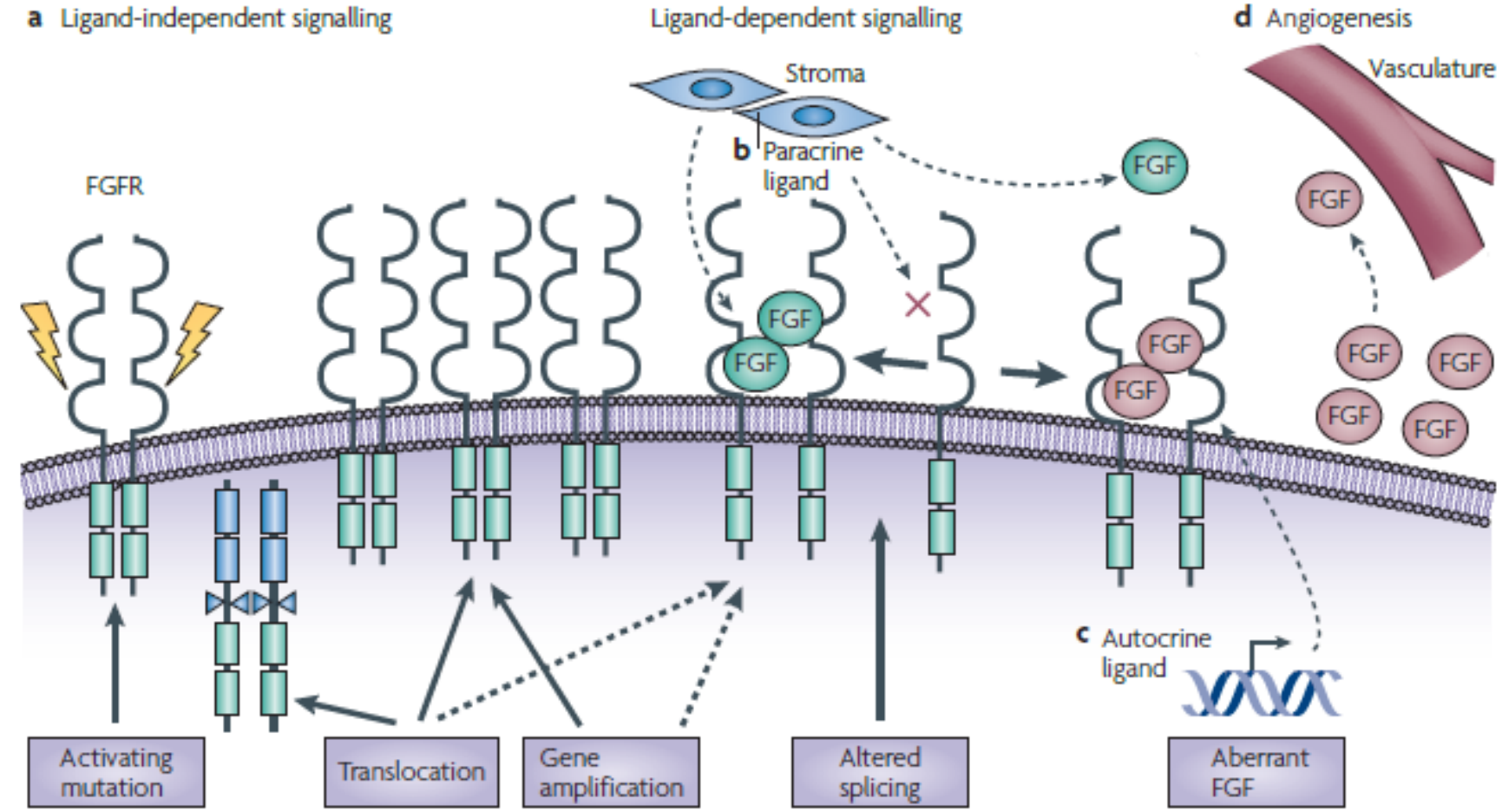
Kelleher et al, Carcinogenesis 2013

FGFR pathway: Oncogenesis by implication



Turner and Grose, Nat Rev Cancer 2010

Primary aberrations in lung cancer: FGFR1 amplification

Table 1. Common FGFR genomic deregulations in solid tumors

Aberration		Tumor	Prevalence (%)
FGFR1	Amplification	Breast (hormone receptor positive)	10
		Lung (squamous cell carcinoma)	10–20
		Lung (small cell)	6
		Head and neck (squamous cell carcinoma)	10–17
		Esophageal (squamous cell carcinoma)	9
		Ovarian	5
		Osteosarcoma	5
FGFR2	Amplification	Breast (triple-negative)	4
		Gastric	5–10
	Mutation	Endometrial	12
FGFR3	Mutation	Bladder (nonmuscle invasive)	50–60
		Bladder (muscle-invasive)	10–15
	Translocation	Bladder (muscle-invasive)	6
		Glioblastoma	3–7
FGFR4	Amplification	Colorectal	5
	Mutation	Rhabdomyosarcoma	8

Dientsmann et al, Annals Onc 2013

Continuous variables

- Clarification of relevance of ‘positivity’ cutpoint and resulting frequency of ‘positives’ depends on associated endpoint
  - Presence above background
  - Prognosis in resection series
  - Predictive of ‘benefit’ from specific therapy in advanced disease (response, PFS, other)

- For ‘amplification’ – additional methodology issues re distinguishing importance of specific region vs associated regions (high polysomy, amplicon, etc)

Sq NSCLC: Weiss et al

- 155 Sq NSCLC. SNP array analysis. GISTIC algorithm.
- **9.7%** (15/155) 133Kb region 8p12 (at least 4 copies = amplification). Region includes FGFR1.

- 8p12 probe FISH **22%** (34/153) Sq NSCLC (at least 9 copies = amplification)

Weiss et al. Sci Transl Med. 2010 Dec 15;2(62):62ra93.

TCGA: FGFR1 And 11q Amplification Seen Commonly In NSCLC

Tumor type	FGFR1 amp freq	FGFR1 and/or 11q amp freq	n
Lung squamous cell carcinoma	16.9%	34.3%	178
Lung adenocarcinoma *	3.5%	14.3%	230

*Provisional TCGA data.
Amplification determined by Genomic Identification of Significant Targets in Cancer analysis of array CGH and SNP array data in TCGA studies

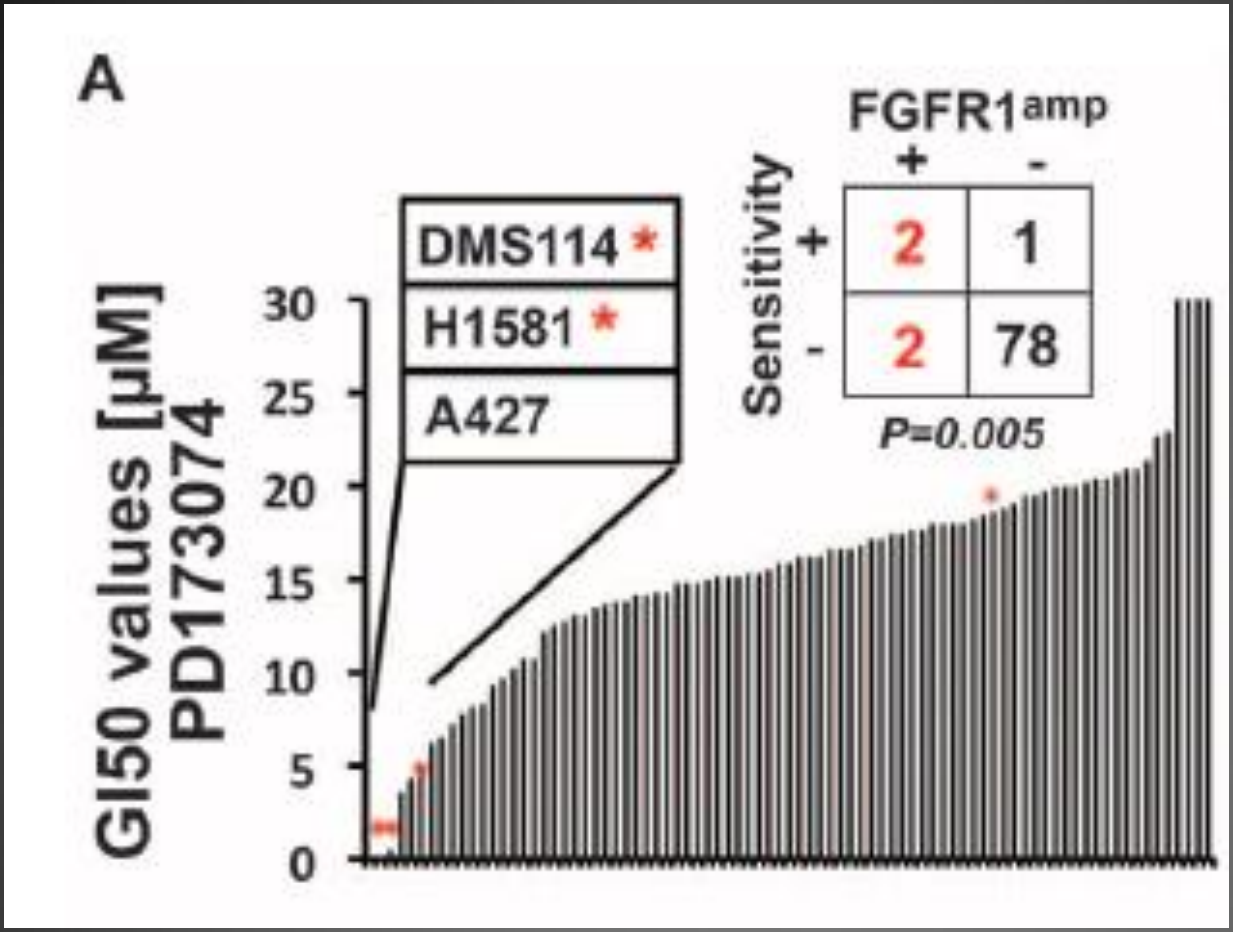
Kinase activity of FGFR inhibitors

	”FGFR inhibitors”			”VEGFR inhibitors”		
	AZD 4547	JNJ 493#	Lucitanib	Nintendanib*	Axitinib#	Pazopanib*
FGFR1	0.3	<1	58	69	218	140
FGFR2	0.2	<1	186	37		
FGFR3	1	1	253	117		130
FGFR4	7	<1	1000	610		800
VEGFR1	87		162	34	1	13
VEGFR2	55		9	21	0.3	12
VEGFR3	35		34	13	0.3	47

* Literature data from EPAR or FDA Pharmacology review
# Published data (poster or article)

Ponatinib

IC_{50} < 10 nM		IC_{50} < 50 nM	
Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
ABL	0.37	BMX	47.2
ABL^{Q252H}	0.44	CSK	12.7
ABL^{Y253F}	0.3	DDR2	16.1
ABL^{T315I}	2	EPHB4	10.2
ABL^{M351T}	0.3	FGFR3	18.2
ABL^{H396P}	0.34	FLT3	12.6
ARG	0.76	JAK1	32.2
BLK	6.1	c-KIT	12.5
EPHA2	2.1	KIT^{D816H}	16
EPHA3	6.7	$PDGFR\alpha^{D842V}$	15.6
EPHA4	1.1	PYK2	35.1
EPHA5	0.69	TIE2	14.3
EPHA7	8.5	TRKA	11.4
EPHA8	2.5	TRKB	15.1
EPHB1	1.2	TRKC	13.2
EPHB2	0.63		
EPHB3	1.1		
FGFR1	2.23		
$FGFR1^{V561M}$	7.3		
FGFR2	1.6		
$FGFR2^{N549H}$	0.45		
FGFR4	7.7		
FGR	0.45		
FMS	8.6		
FRK	1.3		
FYN	0.36		
HCK	0.11		
KIT^{V560G}	0.41		
LCK	0.28		
LYN	0.24		
LYNB	0.21		
PDGFRα	1.1		
$PDGFR\alpha^{V561D}$	0.84		
$PDGFR\alpha^{T674I}$	3		
PDGFRβ	7.7		
RET	0.16		
RET^{V804L}	3.7		
RET^{V804M}	1.4		
c-SRC	5.4		
VEGFR1	3.7		
VEGFR2	1.5		
VEGFR3	2.3		
YES	0.89		



83 lung cancer cell lines

Cellular ATP assay FGFR probe TKI

IC50<1uM = sensitive

DMS114 = SCLC
H1581= large cell
A427 = adeno

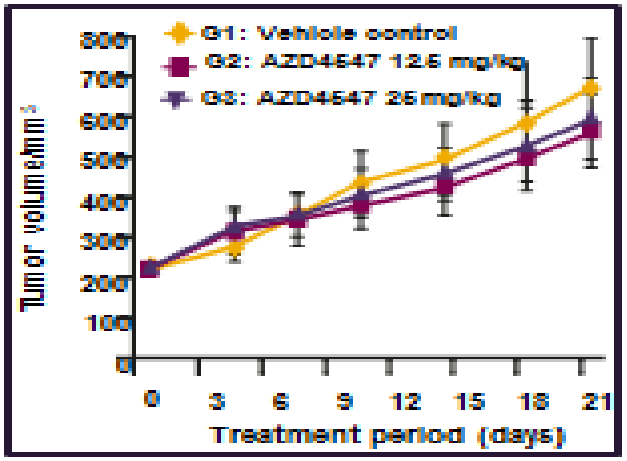
2/3 FGFR inhibitor sensitive lung cancer cell lines 'FGFR1 amp' – 1 of which was squamous (8p12 amp by SNP array = at least 4 copies)

2/4 (50%) 'FGFR1 amp' cell lines sensitive to FGFR inhibition

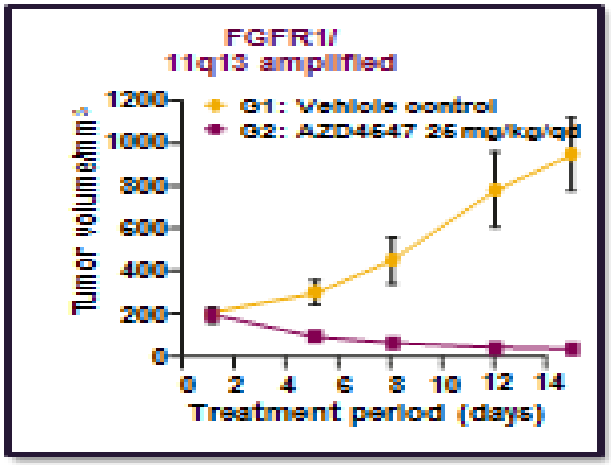
Weiss et al. Sci Transl Med. 2010 Dec 15;2(62):62ra93.

FGFR amplified patient derived explants are sensitive to AZD4547

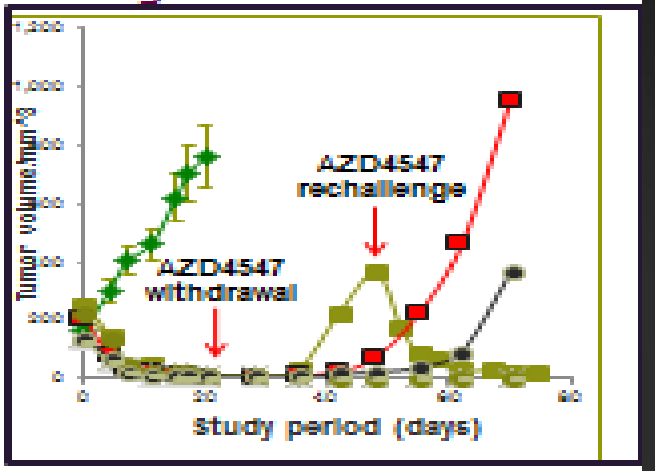
Non- amplified sqNSCLC



FGFR1 amplified sqNSCLC



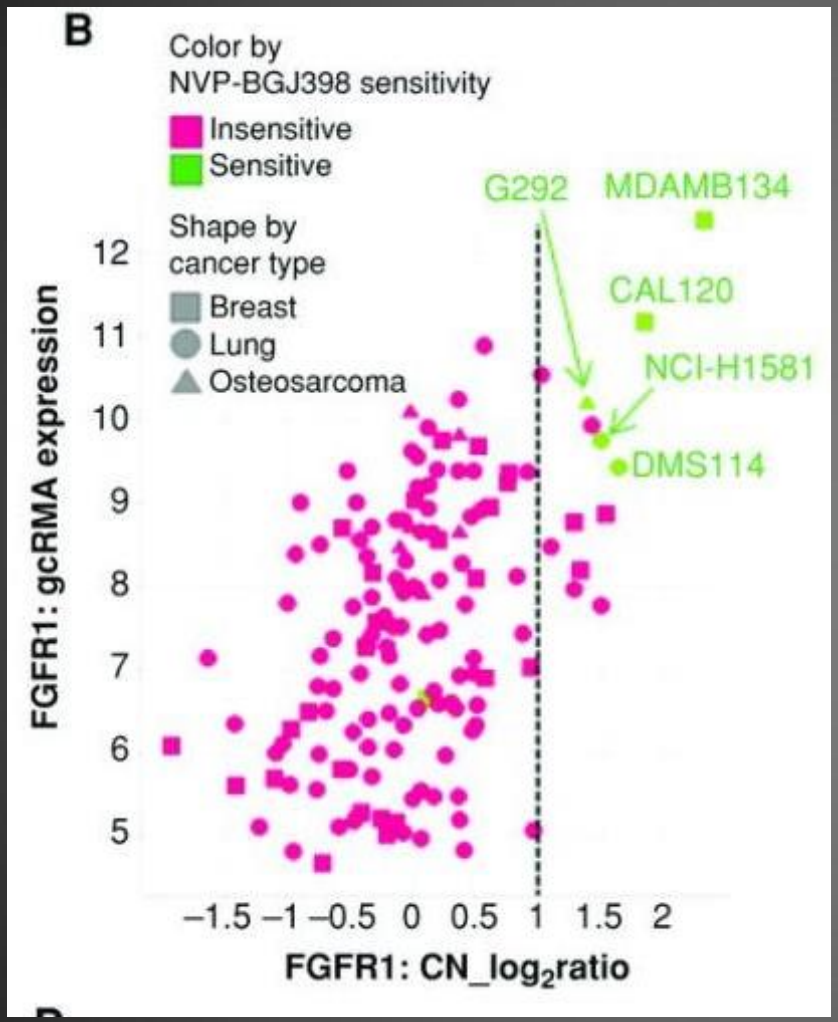
FGFR2 amplified gastric cancer



- Patient derived explant models
- Tumor regressions in *FGFR* amplified models at 12.5 and 25 mg/kg qd
- No efficacy in non-amplified sqNSCLC model

Zhang J *et al. Clin Cancer Res* 2012;18:6658–67
Xie L *et al. Clin Cancer Res. 2013; 19:1-12*

FGFR1 amplification in breast, lung, and osteosarcoma cancer cells is associated with response to NVP-BGJ398.



- *FGFR1* copy number gain defined as $\log_2$ ratio ≥1 (equal to ≥4 normalized DNA copies)
- IC_{50} less than 500 nmol/L were classified as sensitive
- 2/7 lung ca AMP sensitive (28%)
- 2/5 breast ca AMP sensitive (40%)
- 1/1 osteosarcoma AMP sensitive (100%)

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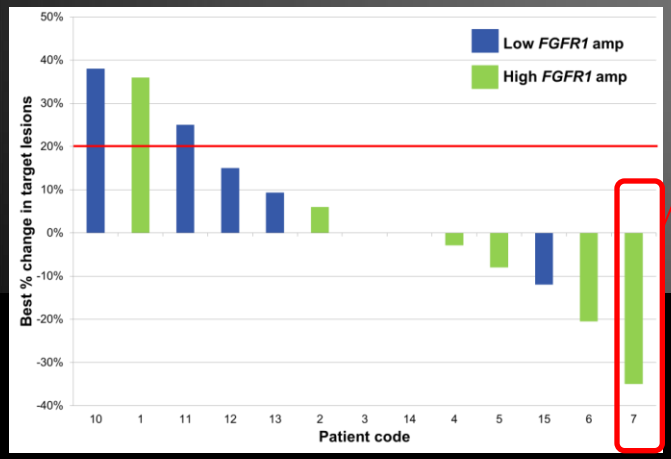
Guagnano V et al. Cancer Discovery 2012;2:1118-1133

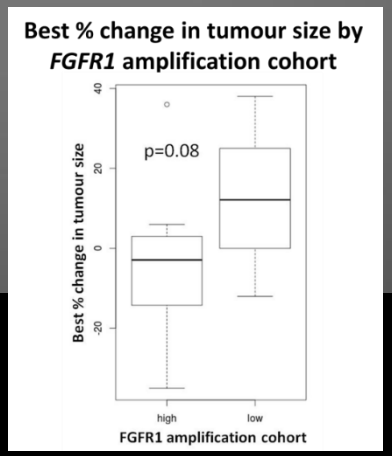
Challenges of moving preclinical data to clinical trial design

- Frequency of ‘amplification’ positivity varies by methodology and cutpoint used – highest rates in squamous cancer
- ‘Positivity’ in small cell line series associated with 28-50% ‘sensitivity’
  - at relatively high IC50s
  - Most preclinical data is growth inhibition. ?best support for ORR as relevant clinical endpoint?
  - Even if shrinkage results - not all sensitivity = objective responses (eg ALK rearranged – 61% ORR to crizotinib even though 90% tumors shrink)

AZD4547 Efficacy in FGFR1 amp Sq NSCLC cohort

- 15 patients treated.
  - 8 patients FISH ratios 2-2.8 (low amplification)
  - 7 patients FISH ratios > 2.8 (high amplification)
- Grade 3 related AEs in 3/15 (20%) (central serous retinopathy, hyponatremia, dehydration) and 3 AE related discontinuations
- 1/14 (7%) evaluable had PR ('high level amp')

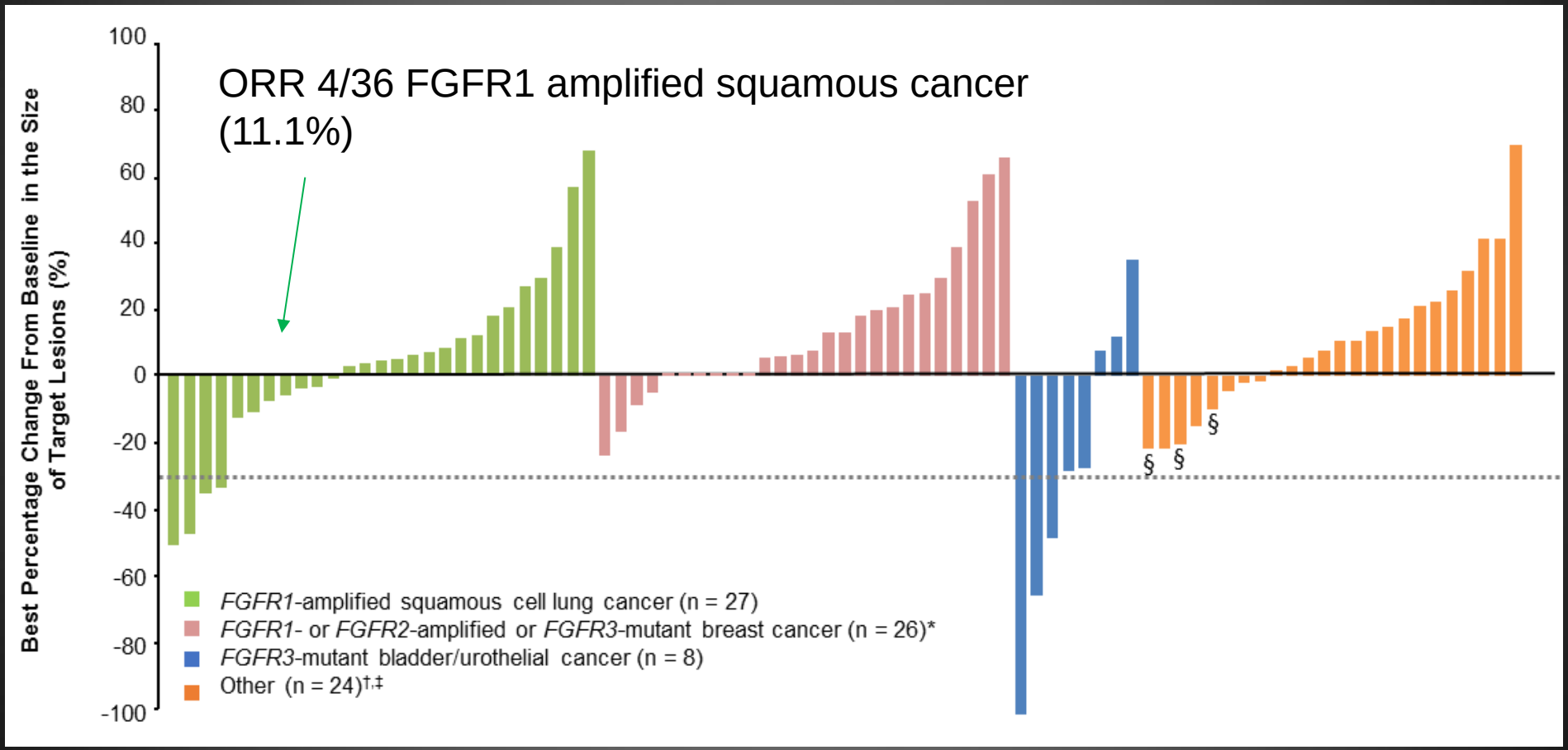




Paik et al, AACR 2014
Paik et al, ASCO 2014

BGJ398 Efficacy

AMP =FGFR1 to chromosome enumeration probe [CEP] 8 [*FGFR1*] ≥ 2.2 or an average *FGFR* copy number ≥ 6 signals/nucleus



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Nogova et al, submitted

Most common AEs (≥20%) and other clinically relevant events

AZD4547 Adverse event	Part A All doses n=43 (%)				Part B&C 80 mg bid n=26 (%)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Constipation	17 (40)	6 (14)	1 (2)	–	9 (35)	2 (8)	–	–
Alopecia	15 (35)	6 (14)	–	–	6 (23)	3 (12)	–	–
Dry mouth	16 (37)	2 (5)	–	–	12 (46)	2 (8)	–	–
Stomatitis	13 (30)	3 (7)	2 (5)	–	4 (15)	3 (12)	1 (4)	–
Hyperphosphatemia*	12 (28)	7 (16)	1 (2)	–	2 (8)	3 (12)	–	–
Nail disorder	9 (21)	8 (19)	2 (5)	–	7 (27)	3 (12)	1 (4)	–
Dry skin	17 (40)	1 (2)	–	–	3 (12)	2 (8)	–	–
Diarrhea	12 (28)	2 (5)	–	–	6 (23)	3 (12)	–	–
Decreased appetite	10 (23)	3 (7)	–	–	7 (27)	3 (12)	–	–
Back pain	9 (21)	3 (7)	1 (2)	–	2 (8)	–	–	–
Vomiting	10 (23)	2 (5)	–	–	6 (23)	2 (8)	–	–
Nausea	10 (23)	1 (2)	–	–	7 (27)	2 (8)	–	–
Fatigue	6 (14)	2 (5)	2 (5)	–	4 (15)	6 (23)	1 (4)	–
Cough	8 (19)	1 (2)	1 (2)	–	–	–	–	–
Asthenia	6 (14)	2 (5)	1 (2)	–	2 (8)	2 (8)	–	–
Retinal pigment epithelial detachment	3 (7)	1 (2.3)	–	–	3 (12)	–	–	–
Other posterior eye signs	–	–	–	–	3 (12)	2 (8)	–	–
Keratitis	1 (2)	3 (7)	–	–		1 (4)	–	–

*Reported as an AE

Tolerability in Sq NSCLC pts?

Treatment-Emergent Adverse Events Occurring in > 30% of All Patients by Treatment								
Adverse Event, n (%)	BGJ398 5-60 mg QD n = 19	BGJ398 100 mg QD n = 6	BGJ398 125 mg Continuous QD n = 57	BGJ398 125 mg 3 Weeks on QD/ 1 Week off n = 40	BGJ398 150 mg QD n = 6	BGJ398 50 mg BID n = 4	All Patients N = 132 All Grades	All Patients N = 132 Grade 3/4
Hyperphosphatemia	**5 (26.3)**	**6 (100)**	**47 (82.5)**	**31 (77.5)**	**5 (83.3)**	**4 (100)**	**98 (74.2)**	**7 (5.3)**
Constipation	1 (5.3)	3 (50.0)	29 (50.9)	18 (45.0)	0	2 (50.0)	53 (40.2)	1 (0.8)
Appetite decreased	2 (10.5)	3 (50.0)	26 (45.6)	17 (42.5)	3 (50.0)	2 (50.0)	53 (40.2)	5 (3.8)
Stomatitis	0	4 (66.7)	26 (45.6)	15 (37.5)	2 (33.3)	1 (25.0)	48 (36.4)	3 (2.3)
Diarrhea	7 (36.8)	5 (83.3)	16 (28.1)	14 (35.0)	1 (16.7)	3 (75.0)	46 (34.8)	0
Nausea	6 (31.6)	4 (66.7)	16 (28.1)	13 (32.5)	3 (50.0)	3 (75.0)	45 (34.1)	2 (1.5)
Fatigue	6 (31.6)	0	16 (28.1)	18 (45.0)	1 (16.7)	2 (50.0)	43 (32.6)	4 (3.0)

Nogova et al, submitted

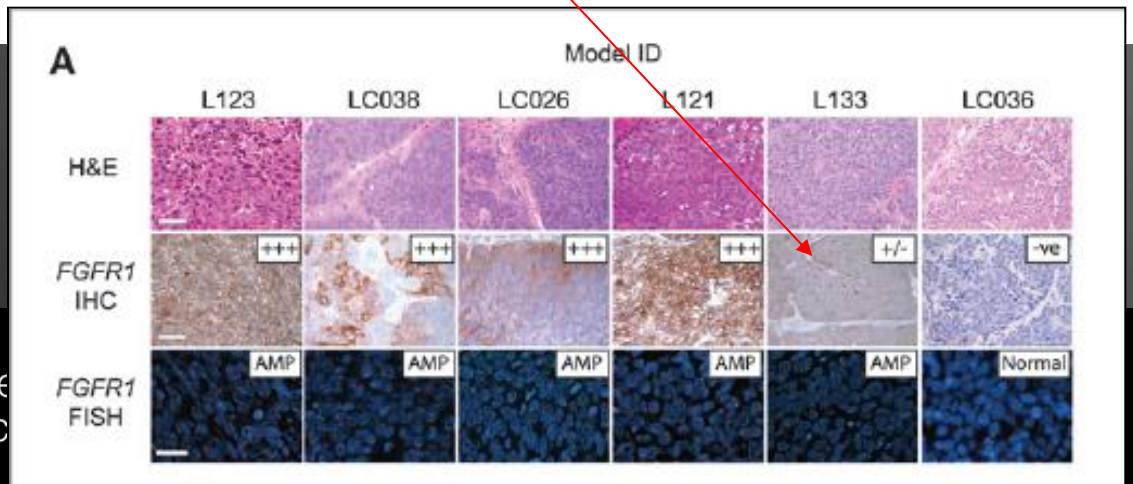
Predictive biomarker performance

IS AMPLIFCATION ALWAYS FUNCTIONAL?: Sq NSCLC PDTX?

	Squamous cell lung primary model ID							
	LC036	LC011	L133	LC022	LC038	LC026	L121	L123
FGFR1 FISH Score (1-6)	1	4	6	5	6	6	6	6
FGFR1 Gene Copy Number (GCN)	1.88	2.58	6	3.94	10	30	10	>10
FGFR1 IHC Score (0-3+)	0	2+	0-1	2+	3+	3+	3+	3+
FGFR1 (WB)								
GAPDH (WB)								
AZD4547 Efficacy at 25mg/kg/qd (% TGI)	18 ($P = 0.25$)	45 ($P = 0.02$)	55 ($P = 0.01$)	69 ($P \le 0.0001$)	94 ($P \le 0.0001$)	134 ($P \le 0.0001$)	190* ($P \le 0.0001$)	199 ($P \le 0.0001$)

kDa: 170, 130, 95, 43

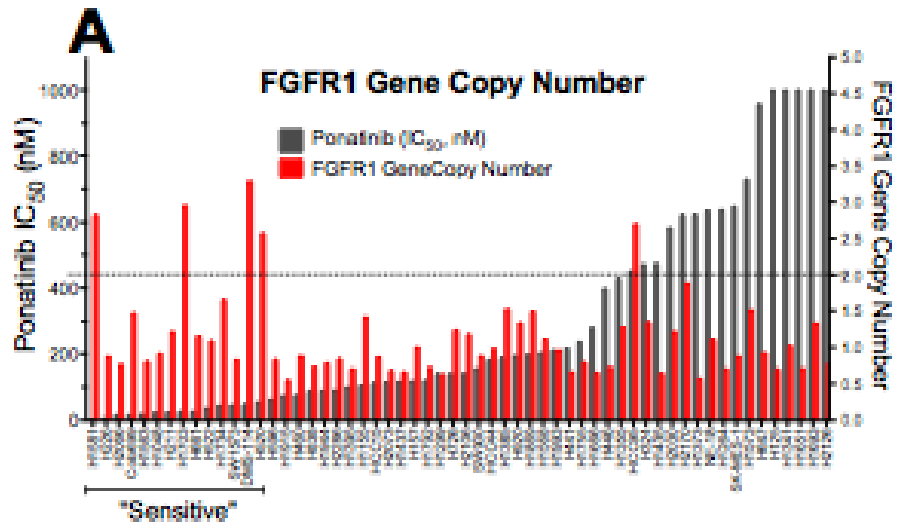
Figure 4. AZD4547 antitumor efficacy correlates well with *FGFR1* gene and protein expression levels. Model summary table displaying *FGFR1* FISH score, gene copy number (GCN), IHC score, protein expression by Western blotting, and antitumor efficacy in response to 2 to 3 weeks, once daily oral AZD4547 treatment (25 or 12 mg/kg). Western blot data were obtained using FGFR1 and GAPDH antisera on fresh tumor fragment lysates. *P* values were calculated using a one-tailed *t* test. *, AZD4547 dosed at 12.5 mg/kg/qd.

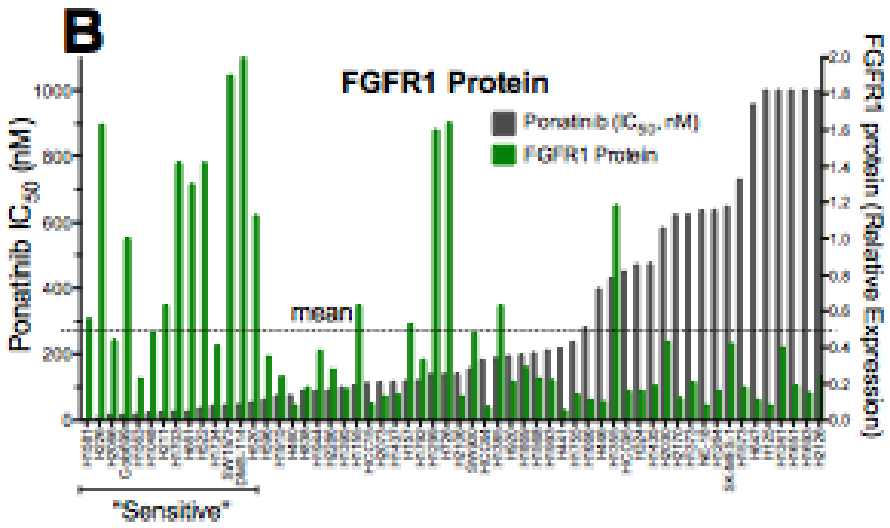


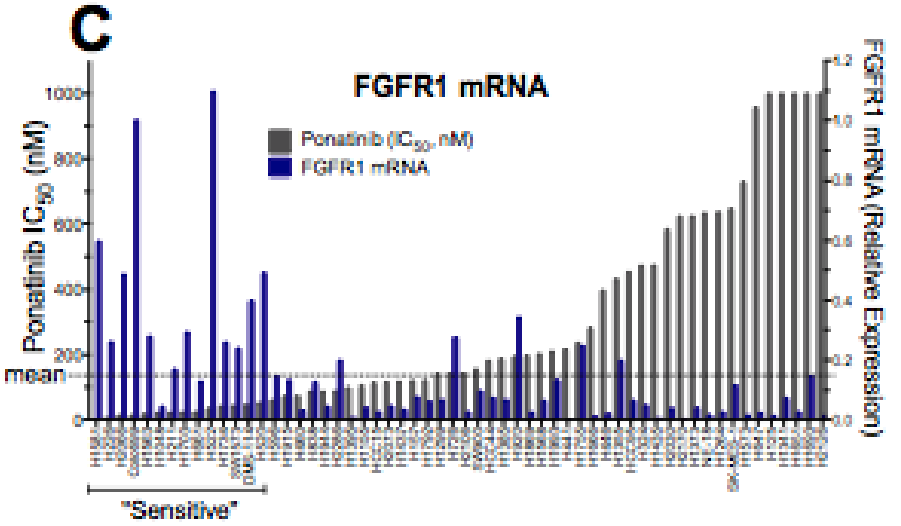
4/5 AMP PDTX models sensitive

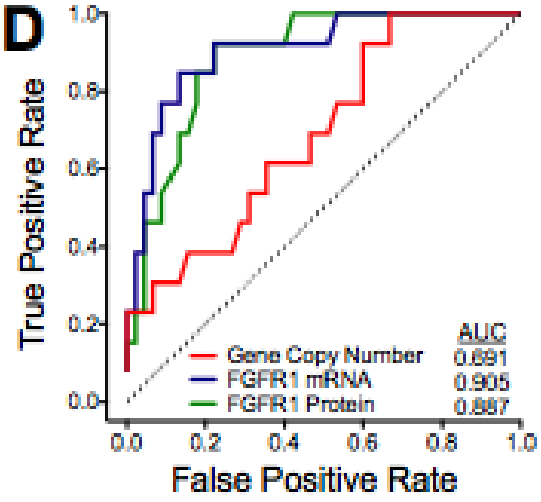
Zhang et al, CCR 2012

Copy number associates with driver state, but is it the driver?





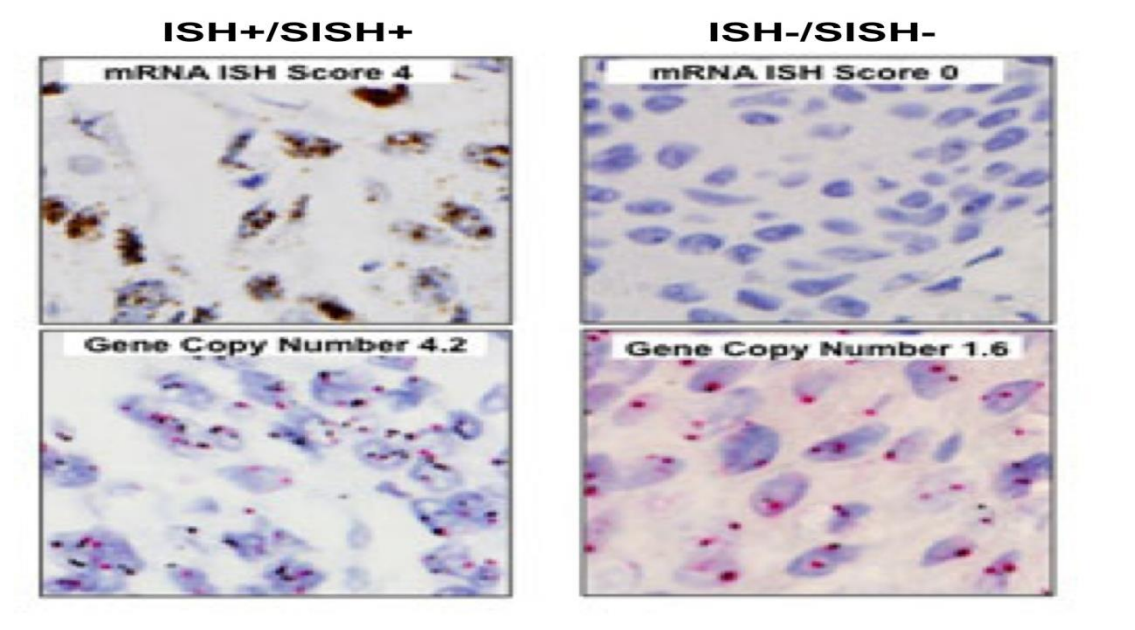


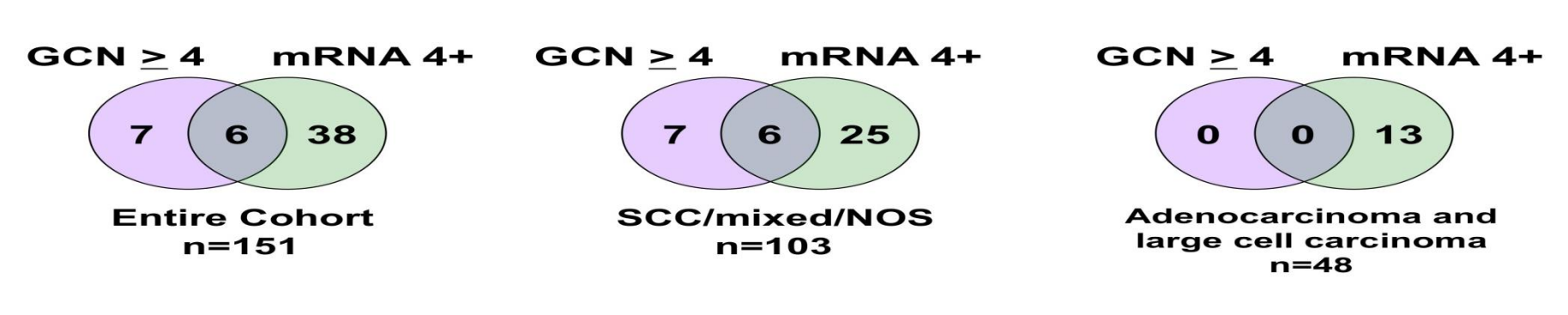


58 lung cancer cell lines

Wynes et al, CCR 2014

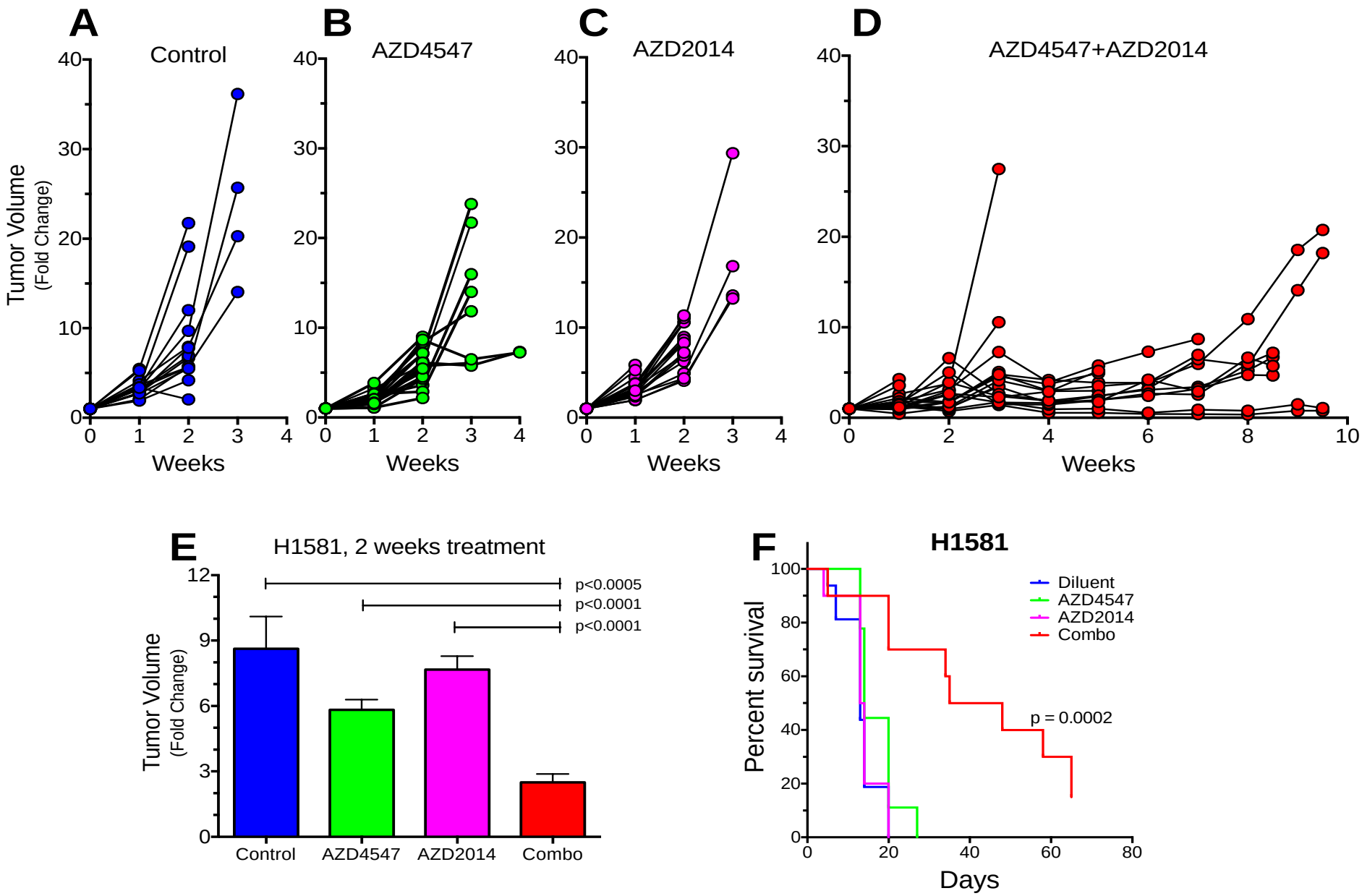
Message vs gene?





Cutpoints from cell line TMA and clinically achievable IC50 for FGFR inhibitor

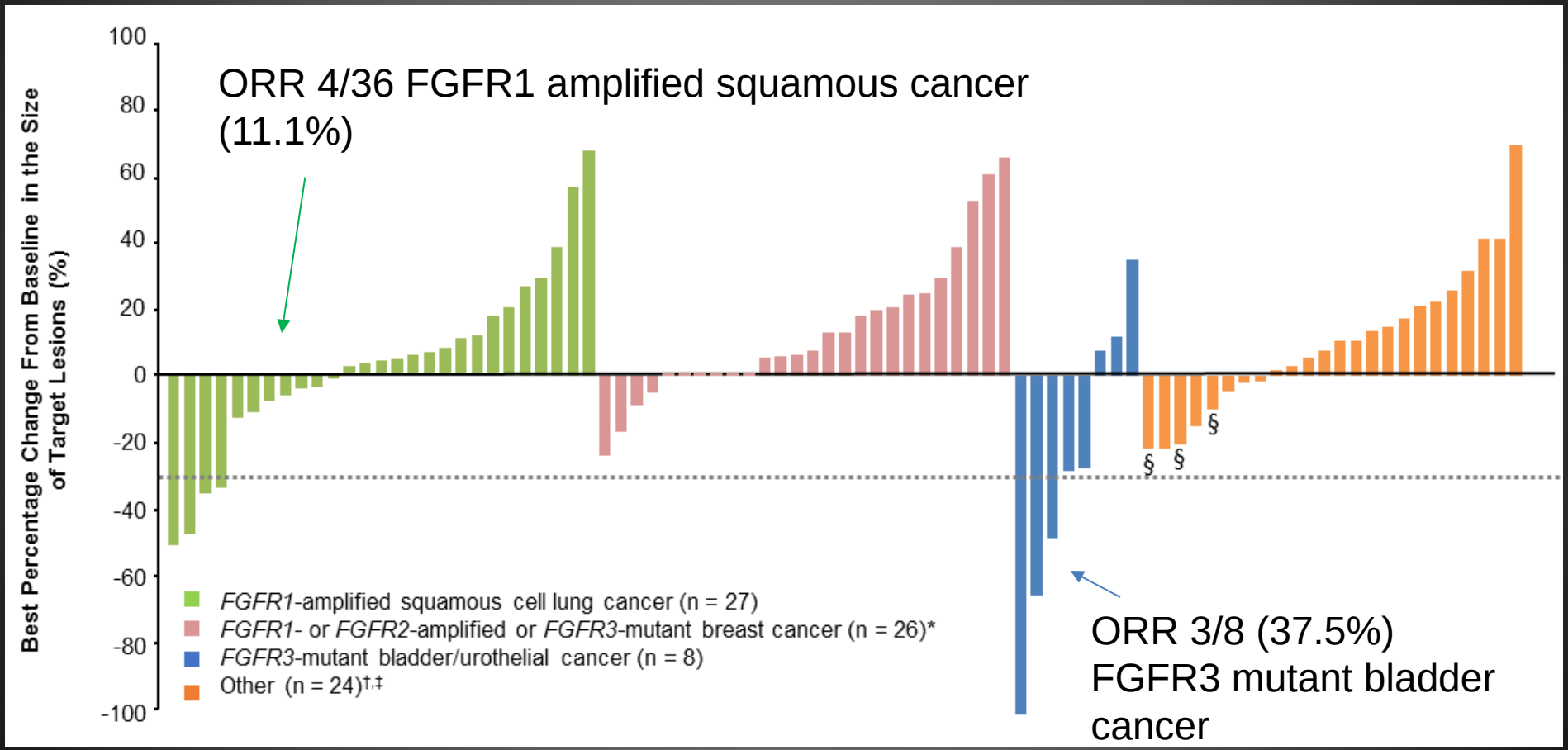
Wynes et al, CCR 2014


A
Control
B
AZD4547
C
AZD2014
D
AZD4547+AZD2014
Tumor Volume
(Fold Change)
Weeks
E
H1581, 2 weeks treatment
p<0.0005
p<0.0001
p<0.0001
Control
AZD4547
AZD2014
Combo
F
H1581
Diluent
AZD4547
AZD2014
Combo
Percent survival
p = 0.0002
Days

Successes?

BGJ398 Efficacy

AMP =FGFR1 to chromosome enumeration probe [CEP] 8 [*FGFR1*] ≥ 2.2 or an average *FGFR* copy number ≥ 6 signals/nucleus



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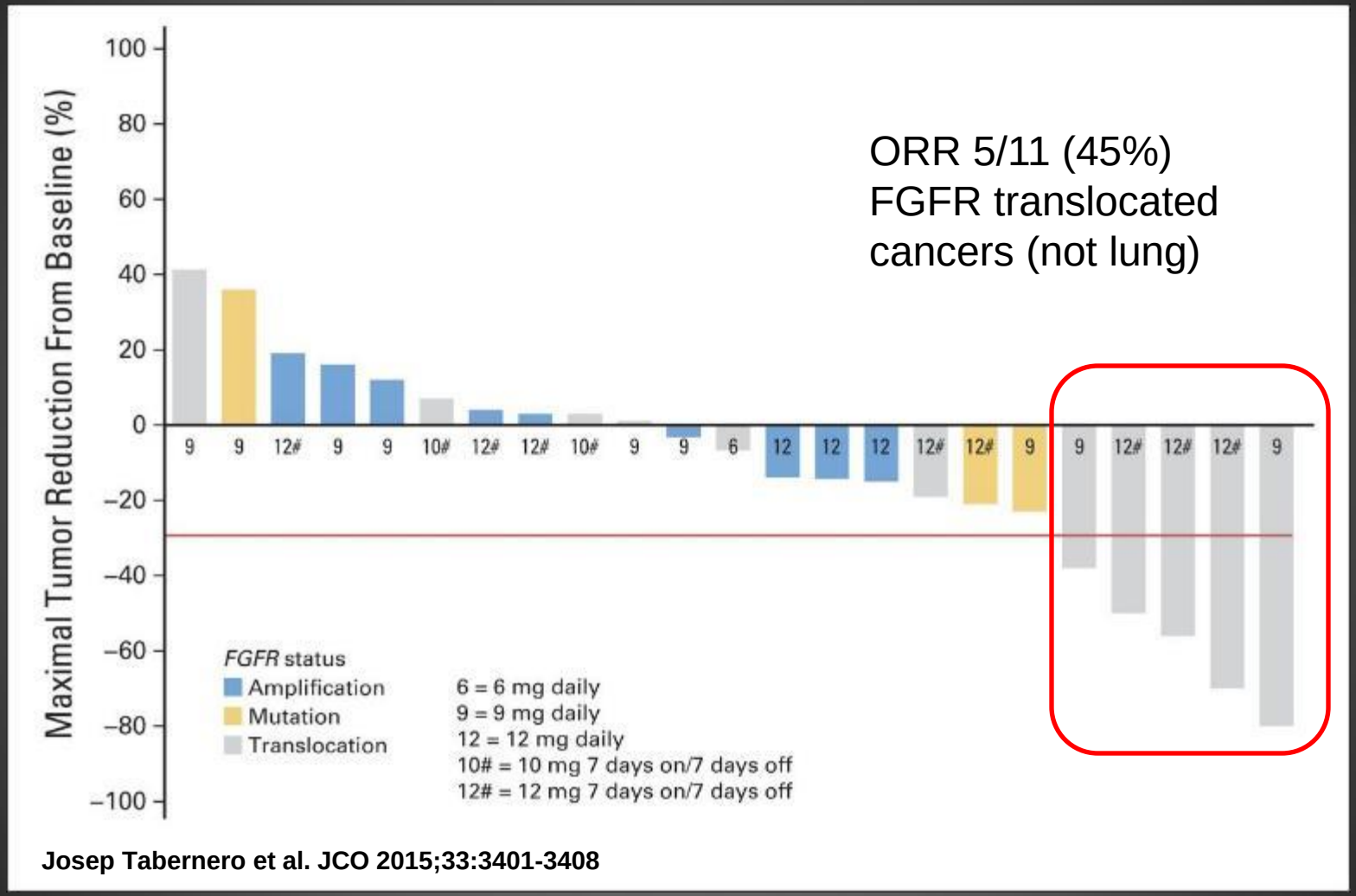
Nogova et al, submitted

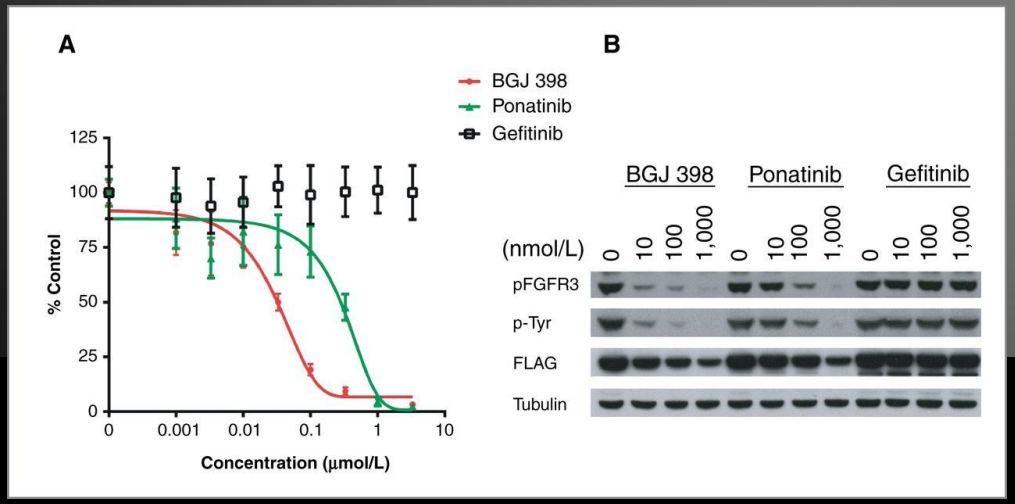
JNJ42756493 Relative change from baseline in target lesion size (at best tumor response).



Josep Tabernero et al. JCO 2015;33:3401-3408

FGFR3 fusions

- 24 lung adenocarcinomas with no known oncogene. NGS
- FGFR3-TACC fusion in 1 patient
- Screen larger cohort and 3 total cases found = 0.3% prevalence adeno
- Sensitive to FGFR TKI in BAF3 model



Marzia Capelletti et al.
Clin Cancer Res 2014;20:6551-6558

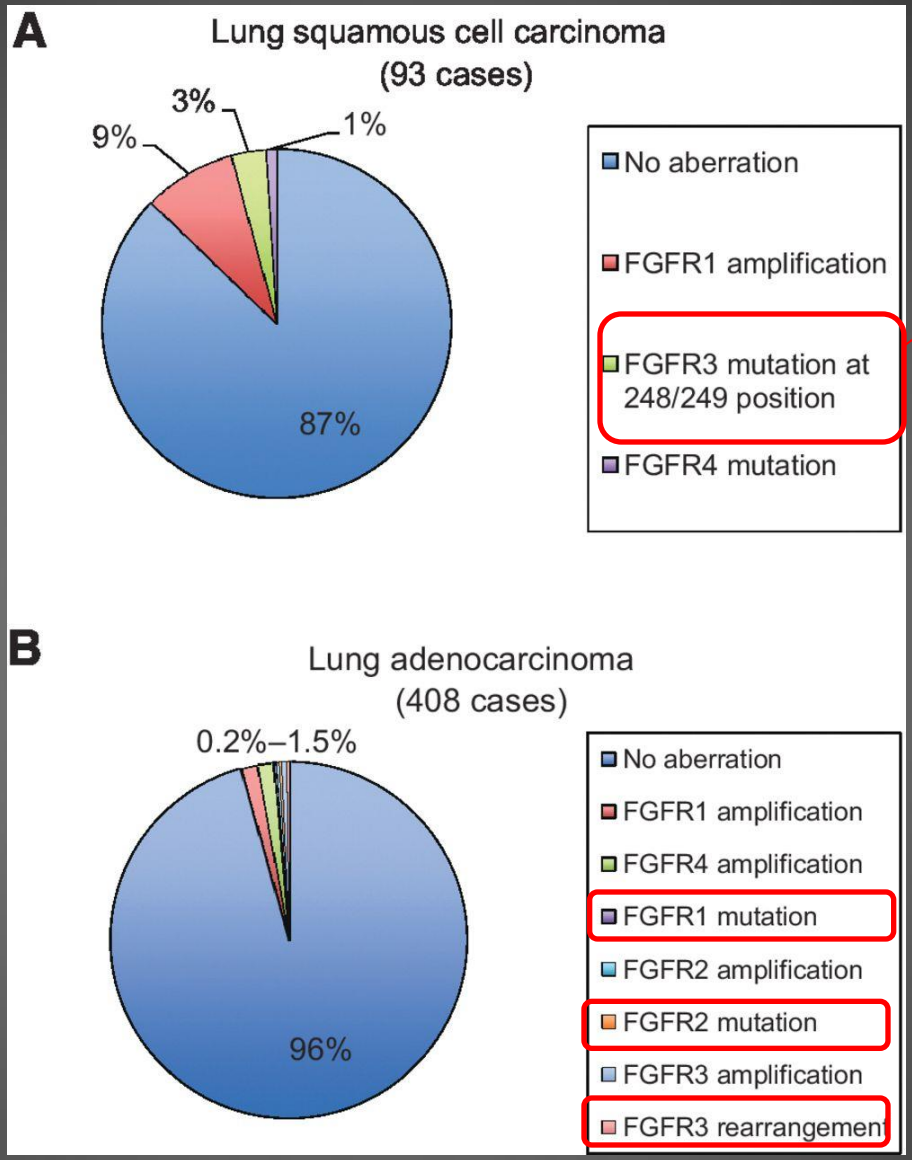
FGFR2 mutations in lung cancer

- 37 year old male never smoker
- NSCLC with prominent glandular differentiation positive staining by IHC for CD56 and TTF-1 and negative for synaptophysin and chromogranin
- Insertion mutation: A266_S267insSTVVGGD
- 21 bp duplication in ECD of FGFR2
- Sensitizing to FGFR TKI in vitro
- V rare example? (0/96 NSCLC screened)

Tanizaki et al, Cancer Research 2015

Relative frequencies of FGFR aberrations in non–small cell lung carcinoma.

Predictive significance of specific mutations and rearrangements currently variably known



3% squamous FGFR3 mutant partial overlap with driver bladder mutations

Mutations and rearrangements exist in adeno but v rare

NB
≥6 copies = gene amplification

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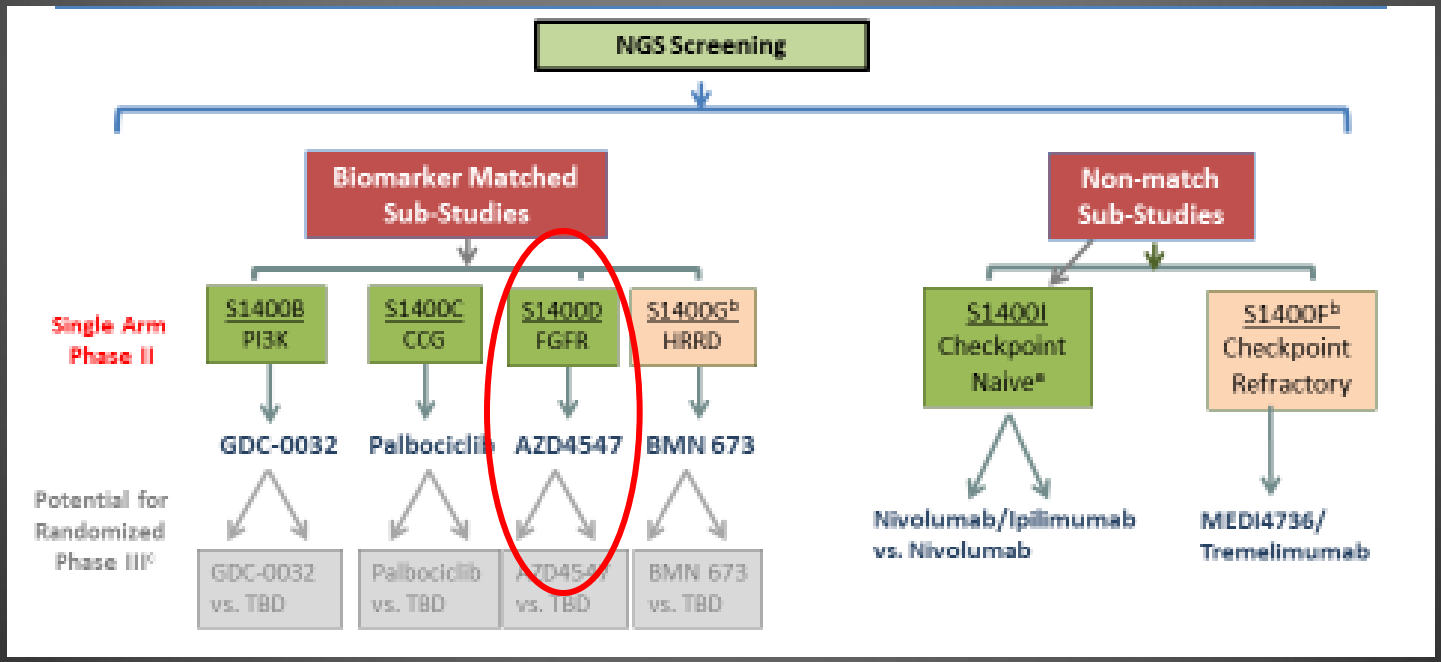
Teresa Helsten et al. Clin Cancer Res 2016;22:259-267

AZD4547: Clinical Development in NSCLC

- AZD4547 is being investigated in a phase II/III trial as part of the Master Lung Protocol in FGFR amplified, mut+, gene fusion+ setting.



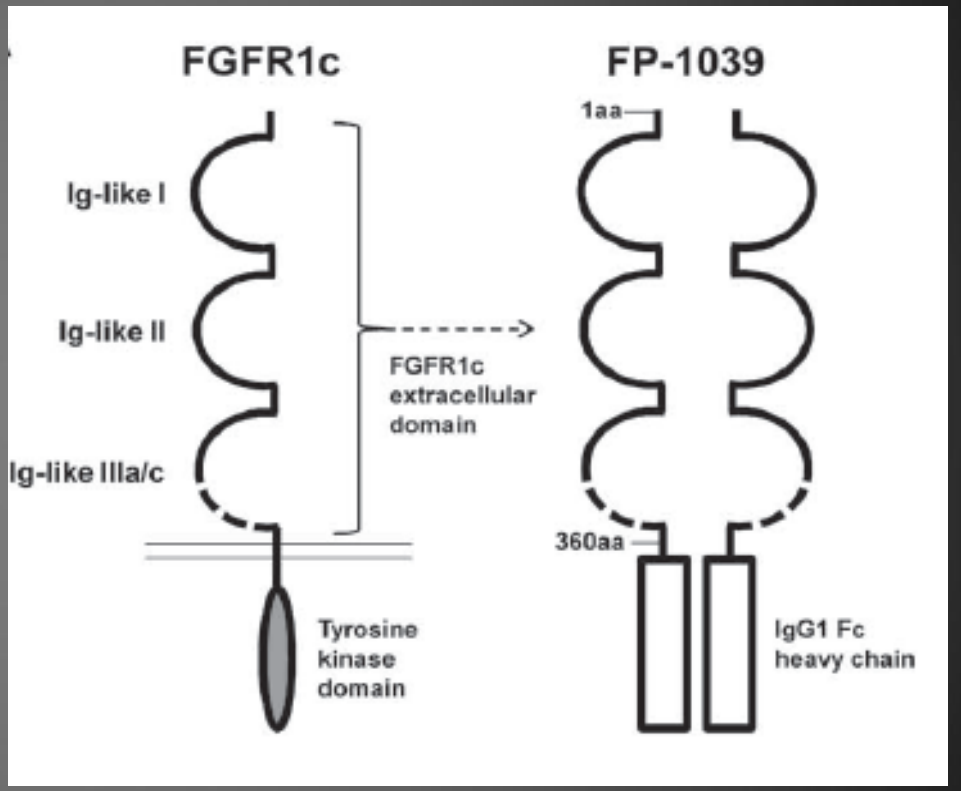
New approaches?

Ongoing directions for FGFR pathway inhibition

- Improved tolerability (drug/dosing/schedule) in tumors with proven drivers (eg FGFR mutations, gene fusions)

FP-1039/GSK3052230 “GSK230”

- FP-1039, also known as HGS1036 or GSK3052230 “GSK230”
- Soluble decoy receptor
- ECD of FGFR1c linked to the hinge and Fc regions of human IgG1
- Binds to certain FGF ligands and prevents the activation of their cognate receptors (potential for ligand dependent cancers)
- Little or no affinity to hormonal FGF (potential for better therapeutic window)



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Harding TC Sci Transl Med 2013; 5: 178ra39

Ongoing directions for FGFR pathway inhibition

- Improved tolerability (drug/dosing/schedule) in tumors with proven drivers (eg FGFR mutations, gene fusions)
- Improved identification of FGFR dependent cases responsible for rare responses in non-mutated/gene fused tumors

Ongoing Ponatinib IITs in FGFR NSCLC

A Phase II Study of Ponatinib in Cohorts of Patients With Lung Cancer Preselected Using Different Candidate Predictive Biomarkers (RET and FGFR)

Clinical Trial Schema:

Acquire tumor block/slides – confirm histology, confirm not EGFR Mt or ALK+ (if adenocarcinoma), confirm adequate for molecular testing

Prospectively screen *FGFR1* mRNA ISH and *FGFR1* copy number SISH all patients

NB FGFR cohorts All histologies

- *FGFR1* ISH+ve, SISH+ve
- *FGFR1* ISH+ve, SISH-ve
- *FGFR1* ISH-ve, SISH+ve
- *FGFR1* ISH-ve, SISH–ve 'double negative'

- Trial uses initial FGFR1 entry cutpoints set by cell line TMA, IC50 and predicted achievable Cmin with ponatinib at 45mg
- Iterative design, adding new cohorts with modified entry cutpoints as data emerge

Summary

- Clinical responses to FGFR inhibitors have been seen in lung cancer
- Preclinical and clinical data suggest FGFR1 amplification inadequate as predictive biomarker
- Mutations and translocations may be highly predictive but are rare in NSCLC
- True basis of sensitivity in FGFR1 amplified cases remains under exploration
- Drug tolerability will need to be addressed for chronic dosing in sensitive cases

Questions?

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