



GARY SPITZER MD

DIRECTOR CLINICAL VALIDITY AND CLINICAL UTILITY

MOLDX : PALMETTO GBA

CONFLICTS OF INTEREST

HAVE CONSULTED FOR TROVAGENE AND INIVATA

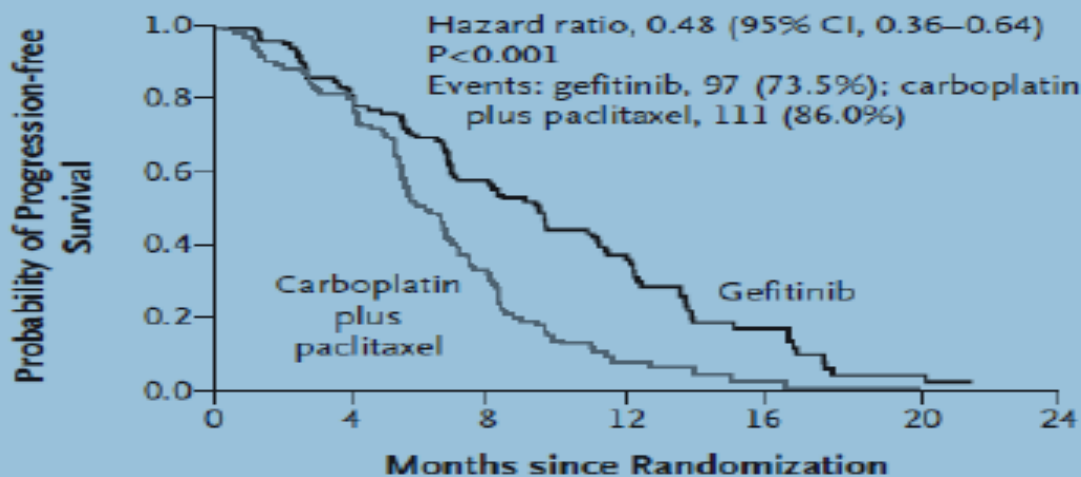
The background is a dark teal gradient. In the corners, there are decorative white line-art elements resembling circuit boards or neural networks, with lines connecting to small circles.

WE HURT THE PATIENT IF WE GIVE A TARGETED
DRUG WHEN THERE IS NO TARGET
DUE TO EITHER A
BIOLOGICAL OR TECHNICAL FALSE POSITIVES
THESE ARE DANGEROUS

**UPFRONT USING AN EGFR WITHOUT THE MUTATION 60% PROGRESSED BY FIRST VISIT
SEE ARROW**

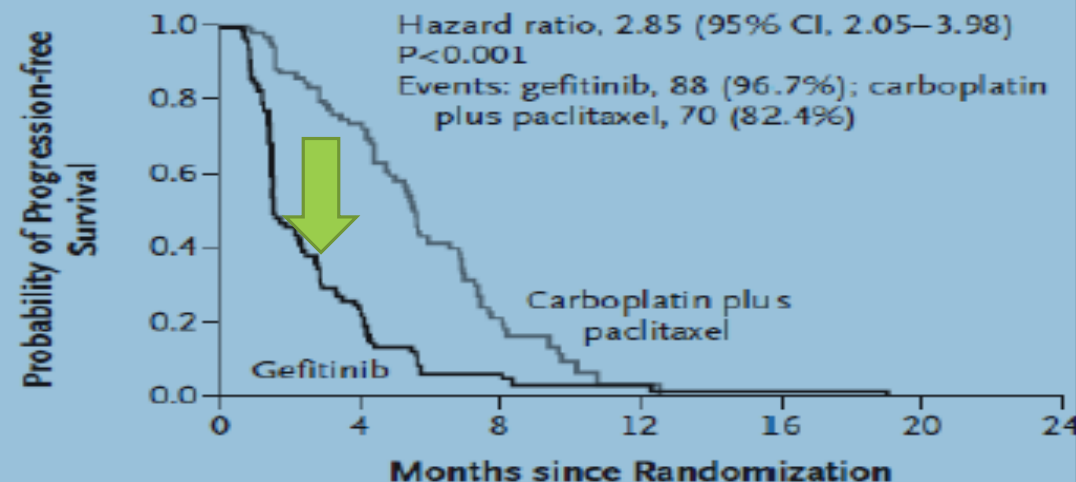
IPASS: gefitinib benefit dependent on EGFR mutation status

EGFR mutation positive



No. at Risk	0	4	8	12	16	20	24
Gefitinib	132	108	71	31	11	3	0
Carboplatin plus paclitaxel	129	103	37	7	2	1	0

EGFR mutation negative



No. at Risk	0	4	8	12	16	20	24
Gefitinib	91	21	4	2	1	0	0
Carboplatin plus paclitaxel	85	58	14	1	0	0	0

FIRST

HAVE A ENOUGH SAMPLES TESTED FOR ACCURATE DETERMINATION OF PERFORMANCE

- WITH A FOUNDER MUTATIONS MY EXPECTATION WOULD BE CLOSE TO 99-100% FOR SPECIFICITY
- AND SENSITIVITY DETERMINATION THAT HAS AT THE MOST A 10% CONFIDENCE INTERVAL
- IF YOU DO NOT HAVE FALSE POSITIVES AND THE CONCORDANCE IS WITH A VALIDATED ASSAY WE DO NOT NEED TO DO PFS
- THE TWO APPROVED ASSAYS WERE TESTED ON 400+ SPECIMENS

APPROVED TESTS FOR FOUNDER MUTATIONS HAVE A HIGH SPECIFICITY AVOIDING HARM TO PATIENT: PLASMA THERASCREEN APPROVED IN EUROPE : 1 / 547 POSITIVE IN PLASMA ALONE <1%

Table 4. Exploratory Biomarker Objective 1 data

Tumour and plasma 1 – screened patients evaluable for both samples (N = 652)					
	Plasma 1 <i>EGFR</i> mutation status (n)				Total
	Positive		Negative		
Adjusted baseline tumour <i>EGFR</i> mutation status, n					
Positive	69		36		105
Negative	1		546		547
Total	70		582		652
	Exon 19 deletions	L858R	L858R and T790M	Negative	Total
Exon 19 deletions	48	0	0	23	71
L858R	0	21	0	12	33
L858R and T790M	0	0	0	1	1
Negative	0	1	0	546	547
Total	48	22	0	582	652
	N		Rate (%)		95% CI
Concordance	652		94.5		92.3–96.0
Sensitivity	105		65.7		55.8–74.7
Specificity	547		99.8		99.0–100.0
PPV	70		98.6		92.3–100.0
NPV	582		93.8		91.5–95.6

ROCHE COBRAS 4/165 POSITIVE 2% IN PLASMA AND TISSUE NEGATIVE

WELL BELOW THE NUMBERS WITH THE T790M RESULTS :76% SENSITIVITY X 10% FREQUENCY X
20% INADEQUATE BIOPSY RATE = 1 TO 2 PATIENTS IN 100 USEFUL TO PREVENT REPEAT BIOPSY

Agreement between Plasma Test & Tissue Test for Detection of EGFR Mutation (with 2.0 mL Plasma Sample)

Rectangular Snip

		cobas® Tissue Test v1		
		EGFR+ (MD)	EGFR(-) (NMD)	Total
cobas® Plasma Test v2	EGFR+ (MD)	161	4	165
	EGFR(-) (NMD)	49	217	266
	Total	210	221	431
With only Valid Result	PPA (95% CI)	76.7% (70.5%, 81.9%)		
	NPA (95% CI)	98.2% (95.4%, 99.3%)		

SO HOW DO WE FEEL ABOUT THE ROCHE ASSAY

- **76% SENSITIVITY X 10% EGFR FREQUENCY X 20% INADEQUATE BIOPSY RATE
= 1 TO 2 PATIENTS IN 100 USEFUL TO PREVENT REPEAT BIOPSY**
- **THIS IS EVEN NOT THE INTENDED USE POPULATION: ALL THESE PATIENTS HAD
ADEQUATE BIOPSY**
 - **WOULD IT PERFORM WITH THE SAME SENSITIVITY IN PATIENTS WITH
INADEQUATE TISSUE**
 - **WOULD BE NICE TO KNOW THE FREQUENCY OF MUTATIONS IN PLASMA
DETECTED IN THIS GROUP COMPARED TO THE STUDIED GROUP**
 - **WOULD BE NICE TO KNOW THE SPECIFIC NATURAL HISTORY OF THE 4 FALSE
POSITIVES**

SECOND

WHAT WE ALSO WANT :TO GIVE THE NODS UP ON CLINICAL UTILITY

- NEW TEST POSITIVE , **BUT** VALIDATED TEST NEGATIVE UNDERSTAND THE **DISCORDANT** RESULT


TISSUE POSITIVE NEW TEST # TISSUE NEGATIVE OLD TEST
PLASMA POSITIVE # TISSUE NEGATIVE

IF SIGNIFICANT DISCORDANCE THE ONLY WAY TO KNOW TRUTH IS NOT TO ASK
WHAT IS TRUTH BUT EXAMINE SPECIFICALLY IF THAT SUBSET BEHAVES LIKE THEY
HAVE THE TARGET

- THERE IS NO NEED TO INCLUDE THE CONCORDANT , THOSE ARE POSITIVE ON A
VALIDATED TEST



CLAIMS WITH T790M PLASMA OR URINE ASSAYS



A HIGHLY SENSITIVE AND QUANTITATIVE TEST PLATFORM FOR DETECTION OF NSCLC EGFR MUTATIONS IN URINE AND PLASMA

KAREN L. RECKAMP ET AL JOURNAL THORACIC ONCOLOGY IN PRESS

WITH THESE TECHNIQUES HAVE THEY REALLY DONE ENOUGH TRUE CONTROLS TO ELIMINATE TECHNICAL FALSE POSITIVES LIKE ROCHE AND QIAGEN DID FOR FOUNDER MUTATIONS : NO THERE ARE VERY SMALL NUMBERS TO CALCULATE TECHNICAL SPECIFICITY AND NO DETAILS OF THE SPECIMENS

CLINICAL EGFR MUTATION DETECTION CUT-OFFS FOR URINE AND PLASMA WERE DETERMINED FOR EACH ASSAY BY ASSESSING THE LEVEL OF NON-SPECIFIC SIGNAL PRESENT, IF ANY, FROM URINE AND PLASMA DNA SAMPLES OBTAINED FROM **54-64 UNIQUE HEALTHY VOLUNTEERS AND METASTATIC PATIENTS WITH NON-NSCLC CANCERS** (APPROXIMATELY 50%/50%). DETECTION CUT-OFFS WERE STANDARDIZED TO 100,000 WT GEQ YIELDING ADJUSTED CLINICAL DETECTION CUT-OFFS OF 5.5, 5.5 AND 12.6 COPIES/105 GEQ FOR EXON 19 DELETIONS, L858R AND T790M, RESPECTIVELY.

SPECIMENS FOR THESE 2 REPORTS ARE FROM THE TIGER X TRAIL WHERE THEY WERE ALL MEANT TO BE T790M + , YET WE HAVE A SMALL NUMBER OF TUMOR T790M- PATIENTS :

- to assess the safety and efficacy of rociletinib in previously treated NSCLC patients known to have the T790M EGFR mutation. BY LOCAL OR CENTRAL LAB
- INTERESTING MUST BE LOTS OF DISCORDANCE BETWEEN LOCAL AND CENTRAL
- Evidence of a tumor with one or more EGFR mutations excluding exon 20 insertion
- Biopsy of either primary or metastatic tumor tissue within 60 days of dosing

AGAIN T- P+ PERCENT OF 12/63 = 19% WHAT IS THE TRUTH

Of 63 patients, 60 had evaluable tissue specimens. Using the tissue result as reference, the sensitivity of EGFR mutation detection in urine was 72% (34/47) for T790M, 75% (12/16) for L858R, and 67% (28/42) for exon 19 deletions. With specimens that met a recommended volume of 90-100 mL, the sensitivity was 93% (13/14) for T790M, 80% (4/5) for L858R, and 83% (10/12) for exon 19 deletions. A comparable sensitivity of EGFR mutation detection was observed in plasma: 93% (38/41) for T790M, 100% (17/17) for L858R, and 87% (34/39) for exon 19 deletions. Together, urine and plasma testing identified 12 additional T790M-positive cases that were either undetectable or inadequate by tissue test.

Karen L. Reckamp, et al J Thor Oncol in press

A decorative graphic on the left side of the slide, consisting of a network of light blue lines and small circles, resembling a circuit board or a data network diagram.

HOW DID 12/60 SPECIMENS FAIL TO
HAVE CONFIRMATION CENTRALLY
WITH THERASCREEN OF THE T790M
STATUS

WE NEED TRANSPARENCY TO
UNDERSTAND THIS. DID THEY HAVE
NGS WITH HIGHER SENSITIVITY

SEE NEXT SLIDE

A Urine vs Tissue

10-100 mL urine

T790M		FFPE Tumor, n			Total
		Positive	Negative	Inadequate	
Urine, n	Positive	34	9	2	45
	Negative	13	4	1	18
Total		47	13	3	63

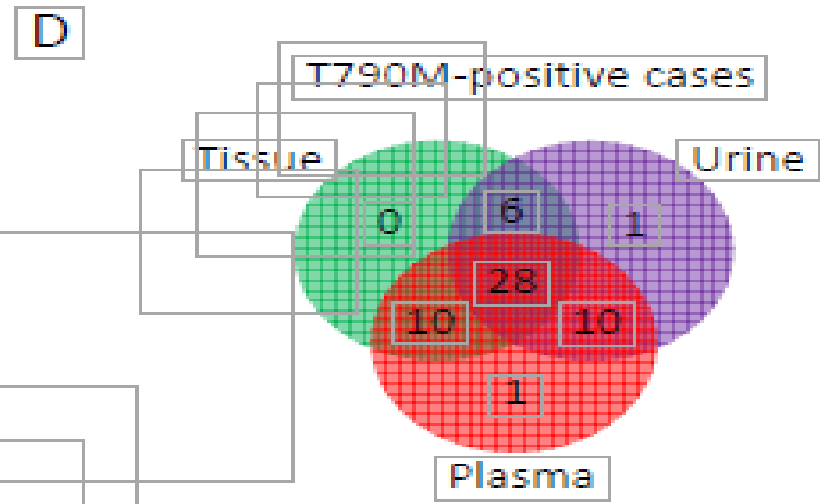
B Plasma vs Tissue

T790M		FFPE Tumor, n			Total
		Positive	Negative	Inadequate	
Plasma, n	Positive	38	9	2	49
	Negative	3	4	1	8
	Failed	3	0	0	3
Total		44	13	3	60

C Urine vs Plasma

10-100 mL urine

T790M		Plasma, n			Total
		Positive	Negative	Failed	
Urine, n	Positive	38	4	3	45
	Negative	11	4	0	15
Total		49	8	3	60



Positive by any one specimen type: 56 of 60 (93%)
 Positive by tissue: 44 of 60 (73%)
 Positive by plasma: 49 of 60 (82%)
 Positive by urine: 45 of 60 (75%)
 Positive by urine and plasma combined: 56 of 60 (93%)
 Negative by any one specimen type: 4 of 60 (7%)

Epidermal growth factor receptor genotyping of matched urine, plasma and tumor tissue from non-small cell lung cancer patients treated with rociletinib

Heather Wakelee,¹ Shirish Gadgeel,² Jonathan Goldman,³ Karen Reckamp,⁴ Chris Karlovich,⁵ Vlada Melnikova,⁶ Jean-Charles Soria,⁷ Helena Yu,⁸ Benjamin Solomon,⁹ Maurice Pérol,¹⁰ Joel Neal,¹ Stephen Liu,¹¹ Mitch Raponi,⁵ Darrin Despain,⁵ Mark Erlander,⁶ Shannon Matheny,⁵ Sergey Yurasov,⁵ D. Ross Camidge,¹² Lecia Sequist¹³

¹Stanford University Medical Center, Stanford, CA, USA; ²Barbara Karmanos Cancer Institute, Detroit, MI, USA; ³UCLA Medical Center, Santa Monica, CA, USA; ⁴City of Hope Comprehensive Cancer Center, Duarte, CA, USA; ⁵Clovis Oncology, Inc., Boulder, CO, USA; ⁶Trovagene, Inc., San Diego, CA, USA; ⁷Gustave Roussy Cancer Center, Villejuif, France; ⁸Memorial Sloan Kettering Cancer Center, New York, NY, USA; ⁹Peter MacCallum Cancer Centre, Melbourne, Australia; ¹⁰Leon Bérard Cancer Center, Lyon, France; ¹¹Georgetown University Medical Center, Washington DC, USA; ¹²University of Colorado, Aurora, CO, USA; ¹³Massachusetts General Hospital, Boston, MA, USA

PRESENTED AT: **ASCO ANNUAL MEETING '16**

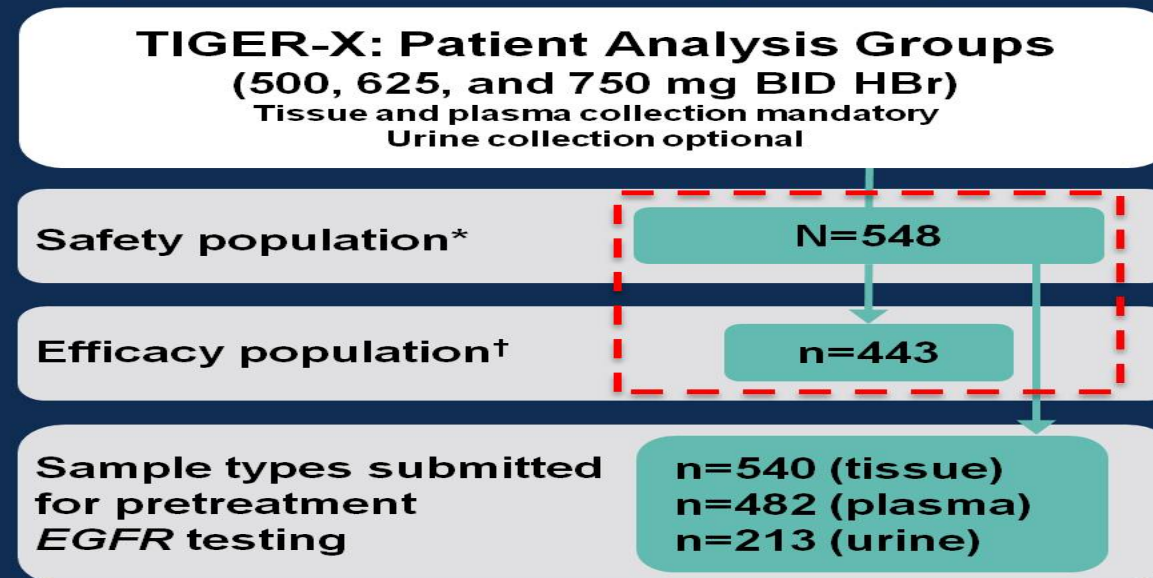
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Abstract 9001

Presented by: Heather A. Wakelee

SAME TRIAL , SAME AUTHORS MUCH LARGER NUMBER OF SPECIMENS WITH SIMULTANEOUS PLASMA AND TISSUE STUDIES . NOTE , ALL THESE STUDIES ARE LIMITED IN TECHNICAL SPECIFICITY BECAUSE OF LIMITED NUMBERS OF EFRG NEGATIVE PATIENTS TO TRULY ASSESS TECHNICAL FALSE POSITIVES . NOW WE HAVE 36 T790M NEGATIVE PATIENTS DISCORDANT RESULTS BETWEEN LOCAL AND CENTRAL LABS

TIGER-X: Safety and Efficacy Population



Data cutoff date: April 15, 2016.

*Includes patients with T790M-negative status by central tissue testing.

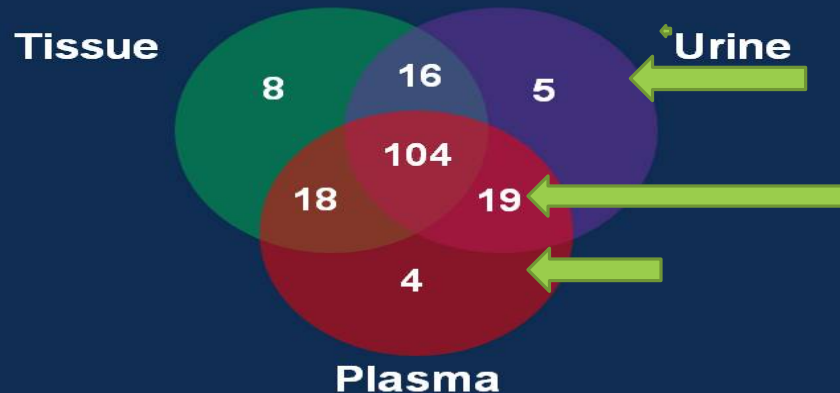
†Patients with centrally confirmed T790M-positive status (determined using Qiagen® *therascreen* test).
HBr, hydrogen bromide.

28/ 181 POSITIVE IN CELL FREE DNA ONLY : 15% : 36 DISCORDANT BETWEEN LOCAL AND CENTRAL LABS

Plasma, Tissue, and Urine Identify Unique and Overlapping Subsets of T790M-Positive Patients

- 181 samples had matched pretreatment T790M results in plasma, tissue, and urine
 - 7 were T790M-negative or inadequate by all 3 sample types (4%)
 - 174 were T790M-positive by at least 1 sample type (96%)

T790M-Positive Cases



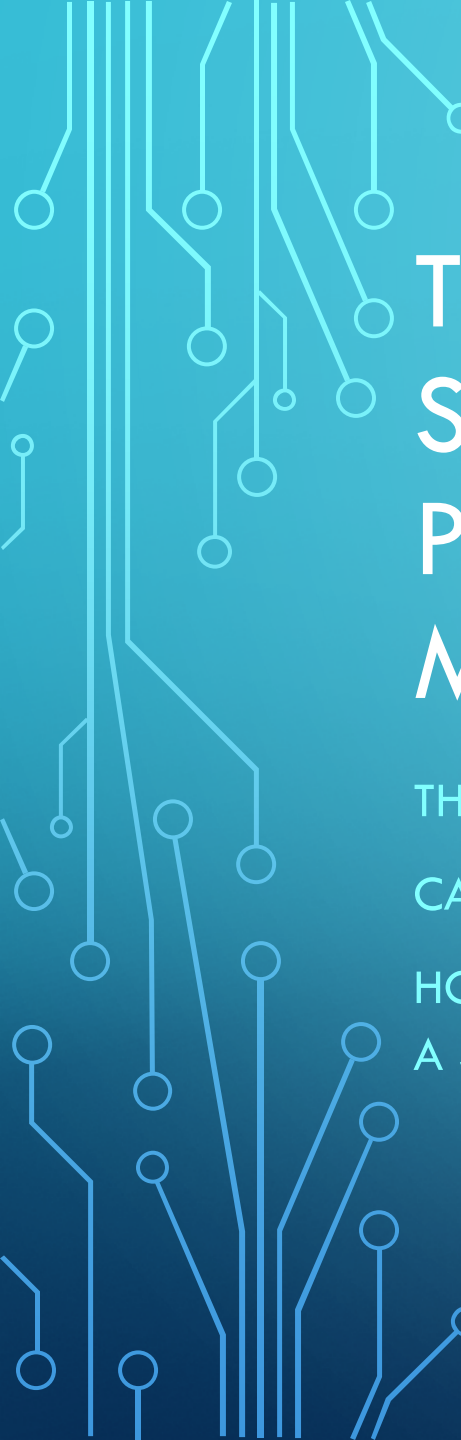
Proportion of patients in diagram not to scale.

- Total positive by tissue: 146 of 181
- Total positive by plasma: 145 of 181
- Total positive by urine: 144 of 181

104 (57%) were positive by all 3 sample types

**PLASMA POSITIVE / TISSUE NEGATIVE IS:
ONLY 1-2% OF PATIENTS USING THE APPROVED
PLASMA TESTS FOR EGFR FOUNDING MUTATIONS
ROCHE AND QIAGEN AND THESE TESTS WERE
DEVELOPED WITH ENOUGH CONTROL SAMPLES TO
DETERMINE SPECIFICITY**

**CAN THESE T790M TESTS CLAIM A SENSITIVITY WHEN
NOT ONE PUBLICATION HAS ADEQUATE OR
APPROPRIATE CONTROLS FOR SPECIFICITY**



THE CLINICAL PROBLEM OR SIGNIFICANCE OF SUB CLONAL POPULATIONS WITH RESISTANT MUTATIONS

THE PROBLEM OF THE PLASMA POSITIVE : TUMOR NEGATIVE

CAN THIS MISLEAD THERAPEUTIC DECISION

HOW DO WE DEFINE THE DETECTION OF A CLONE ONLY IN THE BLOOD REPRESENTS
A SIGNIFICANT TUMOR POPULATION

ASSOCIATION BETWEEN PLASMA GENOTYPING AND OUTCOMES OF TREATMENT WITH OSIMERTINIB (AZD9291) IN ADVANCED NON- SMALL-CELL LUNG CANCER

- Geoffrey R. Oxnard, Kenneth S. Thress, Ryan S. Alden, Rachael Lawrence, Cloud P. Paweletz, Mireille Cantarini, James Chih-Hsin Yang, J. Carl Barrett, and Pasi A. Jänne
- J Clin Oncol 34. © 2016 by American Society of Clinical Oncology In press
- In total, 402 patients were enrolled in the phase I cohorts of AURA but far less than this reported, who progressed on front line EGFR TKI and bio specimens available: population enriched for T790M + patients

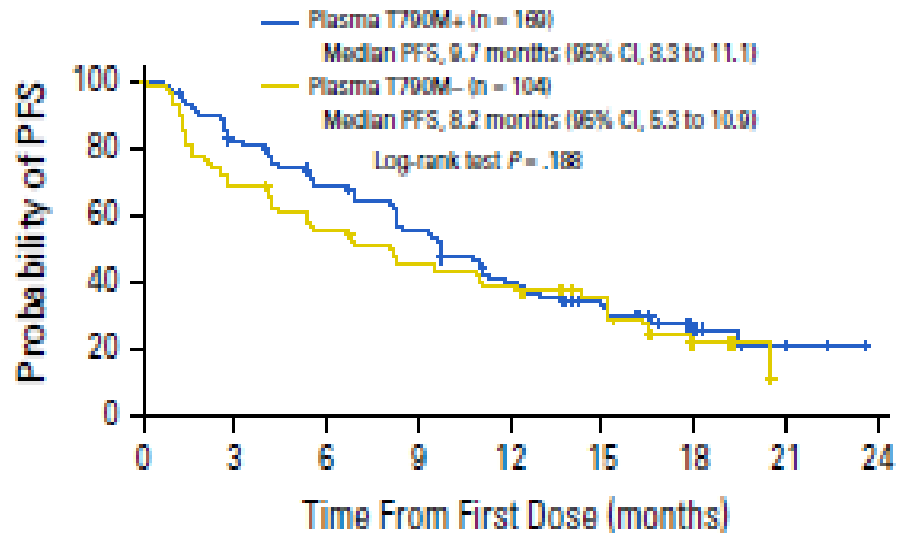
PATIENTS ENROLLED	402
ANALYSIS OF TUMOR GENOTYPE AND OUTCOME	237
ANALYSIS OF PLASMA GENOTYPE AND OUTCOME	271
DIAGNOSTIC COMPARISON OF CENTRAL TUMOR AND PLASMA	216

STUDIES WITH CELL FREE DNA AND THE DETECTION OF T790 M

OXNARD ET AL JCO EPUB JUNE 27 2016

THE PFS ACCORDING TO T790M PLASMA RESULT = NO DIFFERENCE . THIS IS NOT CLINICAL VALIDITY DOES NOT DISTINGUISH 2 GROUPS WITH DIFFERENT OUTCOMES

B



No. at risk

Plasma T790M+	167	130	102	79	58	31	9	2
Plasma T790M-	104	69	54	42	34	25	6	

	POSSIBLE CONFOUNDING VARIABLES	PFS
790M POSITIVE	16% NEG- ON TUMOR	9.7 MOS
790m NEGATIVE	30% POSITIVE ON TUMOR	8.2

THE INSIGNIFICANT DIFFERENCE HERE MAKES YOU SUSPECT THAT THERE ARE PATIENTS WHO HAVE A SHORTER PFS IN PLASMA + THAN EXPECTED WITH THIS DRUG IN THESE CIRCUMSTANCES

LETS BREAK THESE T790M GROUPS DOWN AND SEE IF TUMOR NEGATIVE MAKES A DIFFERENCE

GROUP	
PLASMA POSITIVE	TUMOR NEGATIVE
PLASMA NEGATIVE	TUMOR NEGATIVE
PLASMA NEGATIVE	TUMOR POSITIVE
PLASMA POSITIVE	TUMOR POSITIVE



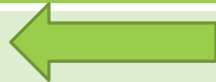
LETS LOOK IN DETAIL AT THE POPULATION WE ARE
CONCERNED ABOUT

DOES PLASMA POSITIVE TUMOR NEGATIVE BEHAVE
DIFFERENTLY THAN PLASMA POSITIVE , TUMOR
POSITIVE?

THE ANSWER IS AN EMPHATIC YES



SPECIFICITY OF THE CELL FREE DNA MEASUREMENT OF T790M

	TUMOR POSITIVE	TUMOR NEGATIVE
PLASMA POSITIVE	111	18 
PLASMA NEGATIVE	47	40

THEREFORE 18 /129 OF PLASMA POSITIVE PATIENTS (14%) WILL BE TUMOR NEGATIVE IF YOU LOOK AT PFS OF ALL PLASMA POSITIVE PATIENTS REGARDLESS IF THIS IS DUE TO BIOLOGICALLY DIFFERENCE OR, THEY ARE TECHNICAL FALSE POSITIVES: SINCE 80% OF THE PATIENTS ARE TUMOR POSITIVE IT IS UNLIKELY THEY WILL CONTRIBUTE SIGNIFICANTLY TO THE PFS EVEN IF THEY DO BADLY . THERE IS NO WAY THEY CAN EFFECT THE MEDIAN .

PFS OF ALL PLASMA POSITIVE PATIENTS. THIS SHOULD NOT BE USED AT THE SURROGATE FOR VALIDITY

AGAIN IN ONLY 14% OF THE PATIENTS ARE TUMOR T790M – AND ALL EGFR + , THEREFORE WE REALLY DO NOT HAVE ANY SPECIFICITY CONTROLS NON EGFR MUTANT NSCLC

COMPARISON OF OUTCOME OF PLASMA POSITIVE VERSUS PLASMA NEGATIVE WHEN TUMOR IS NEGATIVE

	PLASMA + TUMOR NEGATIVE	PLASMA – TUMOR NEGATIVE
RESPONSE RATE	28%	27%
PFS	4.2 MONTHS	2.8 MONTHS
12 MONTH PFS	6% 1 PATIENT 12.25 MOS	20% SEVERAL PATIENTS 18+ MOS

Details of Tumor Negative , Plasma Positive Patients : 4 not confirmed by Orthogonal methods . Highest allele frequency 7% , several less than 0.1 %

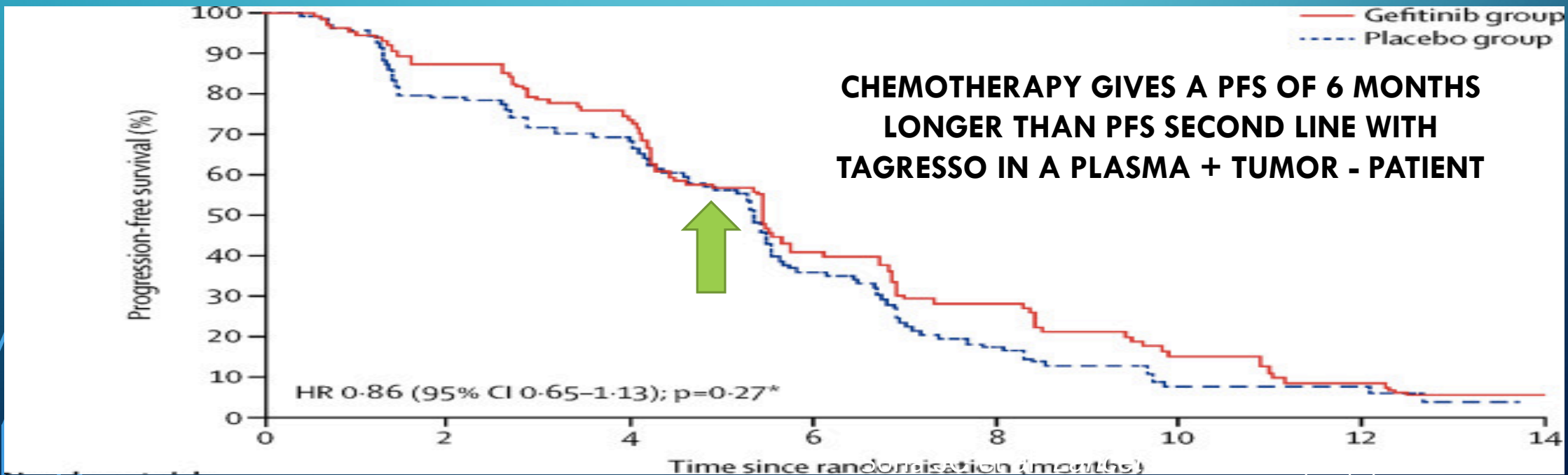
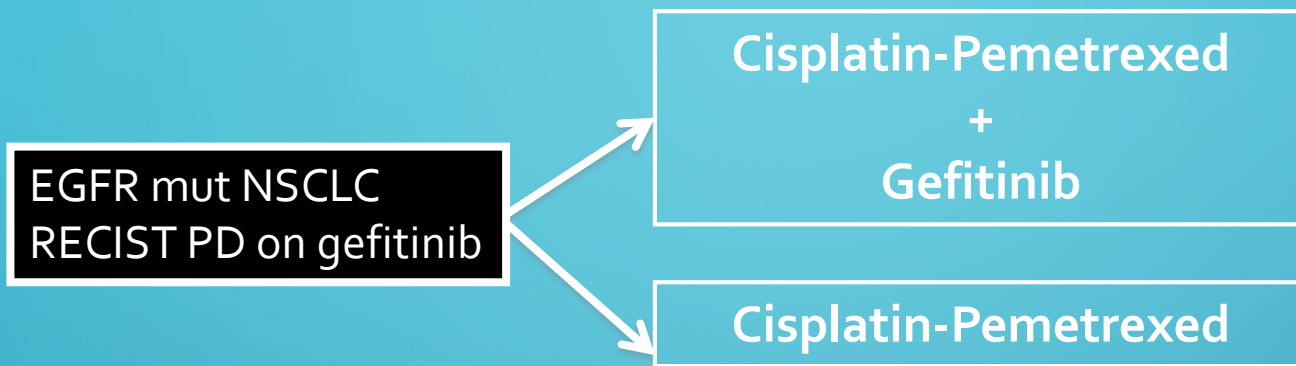
Table A3. Genotyping and Treatment Results for 18 Patients Positive for T790M in Plasma but Negative for T790M in Tumor

Patient No.	Dose	Result of Central Tumor Genotyping for T790M	Result of Central Plasma BEAMing for T790M	T790M AF (BEAMing), %	EGFR Driver	EGFR Driver AF (BEAMing), %	Relative Prevalence Plasma T790M	T790M Detected With Alternative Plasma Assay	Alternative Plasma Assay Used	BOR	Best % Change From Baseline	PFS (month)
12	80 mg	Not detected	Detected	0.19	L858R	3.39	0.06	No	ddPCR	SD	-24.1	12.25*
11	80 mg	Not detected	Detected	0.34	19 del	5.08	0.07	Yes	Cobas	PR	-56.1	11.83
5	80 mg	Not detected	Detected	1.65	19 del	3.42	0.48	Yes	ddPCR	PR	-56.5	9.76*
18	80 mg	Not detected	Detected	0.06	19 del	ND	NA	Yes	ddPCR	SD	-34.1	9.66
1	80 mg	Not detected	Detected	7.05	19 del	34.75	0.2	Yes	ddPCR	PR	-50.6	6.74
14	80 mg	Not detected	Detected	0.09	19 del	11.09	0.01	Yes	ddPCR	SD	-22.2	5.55
7	160 mg	Not detected	Detected	0.64	19 del	ND	NA	Yes	ddPCR	SD	-21.3	5.32
6	80 mg	Not detected	Detected	1.11	L858R	34.14	0.03	Yes	Cobas	SD	-3.8	4.34
17	160 mg	Not detected	Detected	0.07	L858R	0.32	0.23	No	Cobas	PR	-43.4	4.17
4	80 mg	Not detected	Detected	2.04	19 del	18.14	0.11	Yes	Cobas	PR	-62	4.14
9	20 mg	Not detected	Detected	0.45	L858R	28.61	0.02	Yes	Cobas	SD	0	2.73
15	80 mg	Not detected	Detected	0.09	L858R	4.70	0.02	No	ddPCR	SD	-12.1	2.66
16	80 mg	Not detected	Detected	0.08	L858R	0.15	0.55	No	Cobas	SD	-1	2.6
3	40 mg	Not detected	Detected	2.24	L858R*	7.73	0.29	Yes	ddPCR	PD	41.3	1.25
13	40 mg	Not detected	Detected	0.12	L858R	0.37	0.33	Yes	Cobas	PD	-2.2	1.25
10	40 mg	Not detected	Detected	0.34	L858R	2.05	0.17	Yes	Cobas	PD	-20.4	1.05
2	80 mg	Not detected	Detected	3.45	19 del	20.49	0.17	Yes	ddPCR	PD	13.6	1.02
8	160 mg	Not detected	Detected	0.59	19 del	3.14	0.19	Yes	ddPCR	PD	13.3	0.36

Abbreviations: AF, allelic fraction; BOR, best overall response; ddPCR, droplet digital polymerase chain reaction; EGFR, epidermal growth factor receptor; NA, not applicable, ND, not detected; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

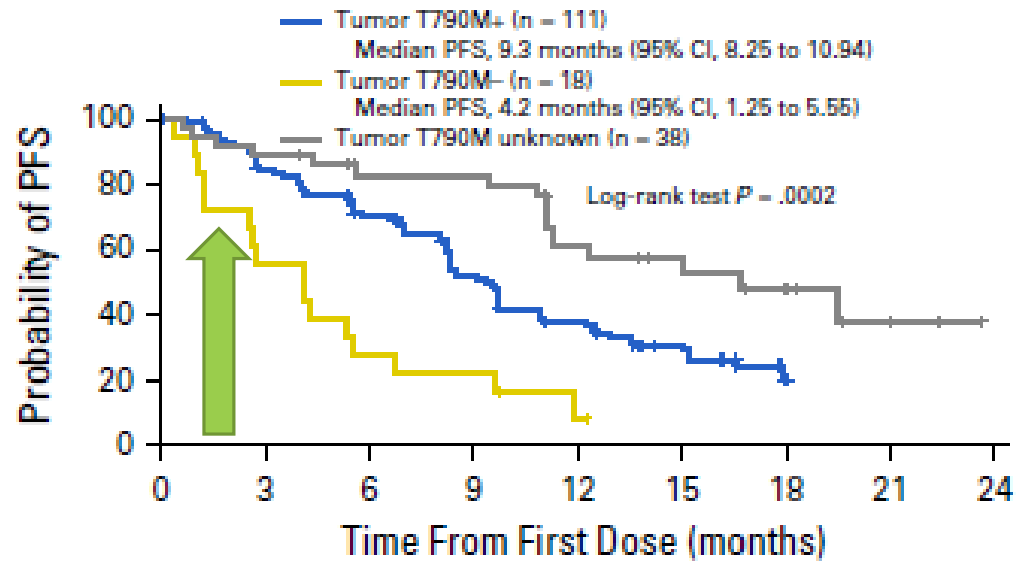
*Subject 3 had discordant tumor and plasma genotyping. Plasma genotyping showed L858R and T790M while tumor genotyping showed G719X and no T790M.

IMPRESS – EVALUATING EGFR TKI CONTINUATION AFTER STARTING CISPLATIN/PEMETREXED FOLLOWING FRONT LINE EGFR FAILURE



SOME CONCLUSIONS FROM THIS SUBSET

- RESPONSE RATE OF 5/16 28% IS NOT SIGNIFICANTLY DIFFERENT THAN THE RESPONSE RATE IN TUMORS NEGATIVE FOR T790M
- THIS DRUG IS NOT SELECTIVE IN PATIENTS WITH THIS MUTATION AND MAY IN FACT ULTIMATELY BE GRADUATED TO FIRST LINE
- THE PFS IS CLEARLY NO WHERE NEAR 9 MONTHS ONLY 4 OF 16 PATIENTS REACHING THIS POINT AND NO PATIENT IS STILL RESPONDING . PFS= 4.2 MONTHS (SEE TABLE)
- THIS IS AGAIN IS REFLECTED IN THE MARKED DIFFERENCE SHOWN IN THE NEXT CURVE : WHERE PATIENTS WITH POSITIVE TUMORS AND PLASMA TESTS CLEARLY DO BETTER THAN IF THE TUMOR IS NEGATIVE , AND NOTE HOW QUICKLY SOME PATIENTS HAVE PROGRESSED BY THE FIRST FOLLOW UP 2 MONTHS OR LESS

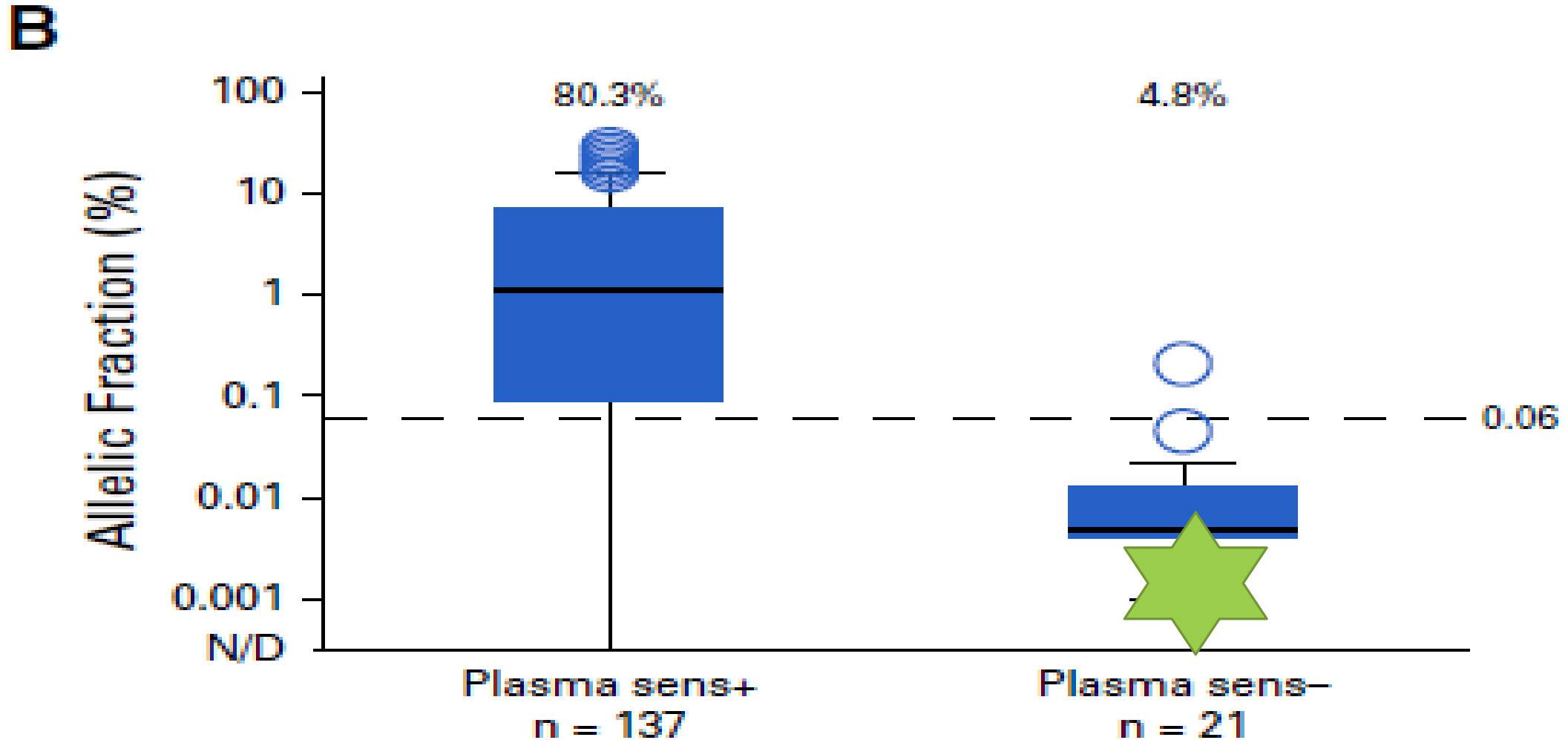
D

	No. at risk	0	3	6	9	12	15	18	21	24
Tumor T790M+	111	88	70	48	33	19	1			
Tumor T790M-	18	10	5	4	1					
Tumor unknown	38	32	27	26	19	12	8	2		

- T- P+, THESE PATIENTS POTENTIALLY COULD DO BETTER WITH CHEMOTHERAPY
- IF WE LOOK AT THE TABLE THEY ALL HAD THE SENSITIZING MUTATION SO THE TECHNICAL FALSE POSITIVE MAY NOT BE SO MUCH IN PLAY
- THIS MAYBE A CONCERNING PROBLEM WITH A NON FOUNDER THAT SMALL CLONES IN THE BLOOD ARE NOT BIOLOGICALLY RELEVANT

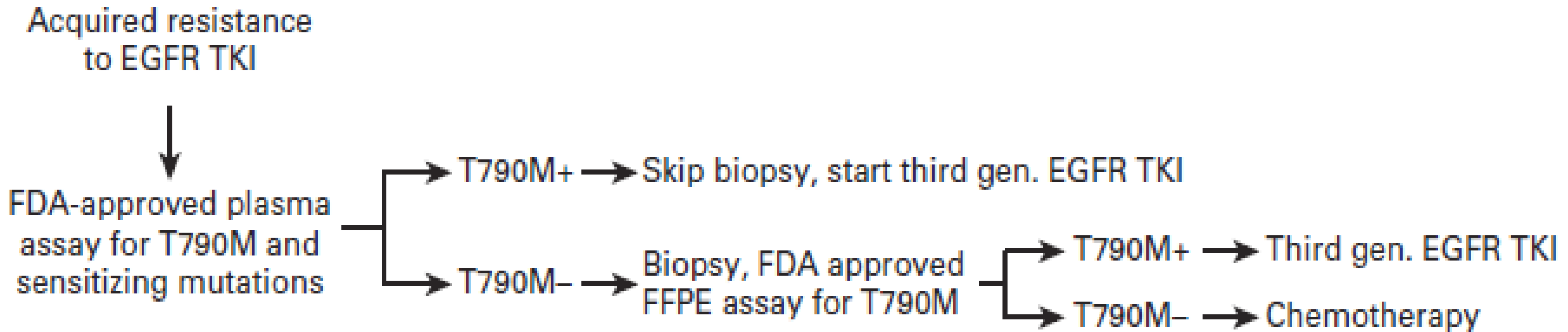
- CAN SOME OF THESE PLASMA T790M PATIENTS BE TECHNICALLY FALSE POSITIVE
- TO QUOTE THE MANUSCRIPT , AMONG PATIENTS WITH T790M POSITIVE ON TUMOR GENOTYPING, SENSITIVITY OF THE PLASMA T790M ASSAY IS LOWER WHERE NO EGFR SENSITIZING MUTATION IS DETECTED IN PLASMA ($P < 0.01$) ,
- YET THE ASSAY FOR THE FOUNDER MUTATIONS IS MORE SENSITIVE , HOW CAN WE PICK UP 21/158 , 13% MORE CALLS WITH A LESS SENSITIVE ASSAY WHICH IF TRUE , SHOULD HAVE IDENTIFIED THE FOUNDER CLONE WITH THE SIMULTANEOUS MORE SENSITIVE ASSAY
- SEE NEXT SLIDE 21 POSITIVE CASES WITH NO FOUNDER MUTATION

21 PLASMA ASSAYS WERE POSITIVE WHEN THE FOUNDING EGFR MUTATION WAS NOT PRESENT YET THE FOUNDING MUTATION ASSAY IS A MORE SENSITIVE ASSAY AND SHOULD BE AT LEAST AT THE SAME ALLELE FREQUENCY ; DOES THIS MAKE SENSE , WHAT SIZE CLONES ARE WE MEASURING?



**PROPOSAL FROM MANY:
IF PLASMA TEST IS POSITIVE, FORGO TISSUE BIOPSY DESPITE
SUGGESTION THAT POTENTIALLY 14/100 PATIENTS THIS MAY
NOT BE OF BENEFIT, AND MAY EVEN BE HARMFUL**

B



IN FACT THE AUTHORS POINT THIS OUT IN THE MANUSCRIPT

Dividing patients with T790M-negative plasma results on the basis of tumor geno-typing results, ORR was higher in patients with T790M-positive tumors (31 of 45; 69%; 95% CI, 53% to 82%) than in patients with T790M-negative tumors (10 of 40; 25%; 95% CI, 13% to 41%; P , .001), as was median PFS (16.5 months v 2.8 months; P , .001; Fig 3C). *Of interest, patients with T790M-positive plasma could also be divided on the basis of tumor genotyping results, with ORR and median PFS higher in those with T790M-positive tumors (69 of 108; 64%; 95% CI, 54% to 73%; PFS, 9.3 months) than in those with T790M-negative tumors (5 of 18; 28%; 95% CI, 10% to 53%; P = .004; PFS, 4.2 months, P = .0002; Fig 3D).*



THE PROBLEM

WHEN MEASURING A SUB CLONE AND NOT A FOUNDER MUTATION HOW DO WE IDENTIFY IT IS THE PREDOMINANT MOST RELEVANT CLONE

EVEN THOUGH A NUMBER OF THESE DISCREPANCIES BETWEEN TISSUE AND CONCORDANT PLASMA SAMPLES MAY BE TECHNICAL , IT MAY REALLY BE BIOLOGICAL

USE OF CELL FREE DNA IN THE RESISTANT SPACE WHERE WE DEAL WITH SUB CLONES MAYBE FRAUGHT WITH DANGER IN MAKING THERAPEUTIC DECISIONS AND DO HARM TO THE PATIENT

REAL CONCERNS ARE EXPRESSED IN THE COLON CANCER SPACE, WHERE THEY PROPOSING MAKING DECISIONS ON LOW ALLELE FREQUENCY OF RAS MUTATIONS

QUESTION

- The frequency of a drug resistance mutation that should necessitate a change of treatment when an alternate therapy exists is unknown. What should be the clinical response when the new clone is 0.1% of tumor cells that is otherwise sensitive to a targeted drug. If the technology is valid a resistant clone is clearly emerging, but in this setting the majority of the tumour presumably continues to be suppressed by use of the current agent. Some BCR–ABL1 kinase domain resistance mutations can be present at low levels before TKI treatment without leading to clinical relapse. Likewise, in patients with AML, the AML–ETO fusion product, which is considered a driver of the disease, can remain detectable at low levels in the blood in patients who have been in complete remission for years.

The background is a solid teal color. In the four corners, there are decorative white line-art patterns resembling circuit traces or neural networks, with small circles at the end of the lines.

FOR A SUBCLONE MUTATION

**SHOW
PLASMA + TISSUE –
PFS**

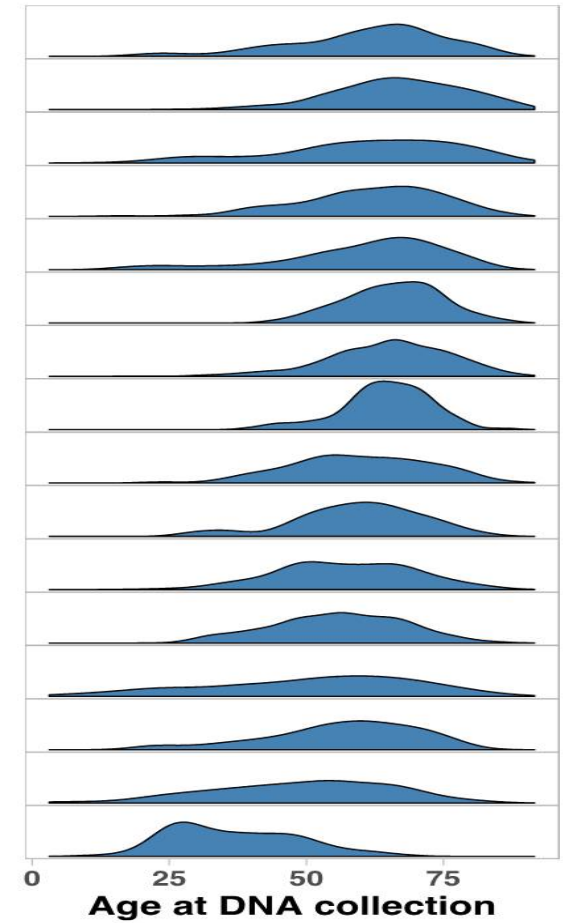
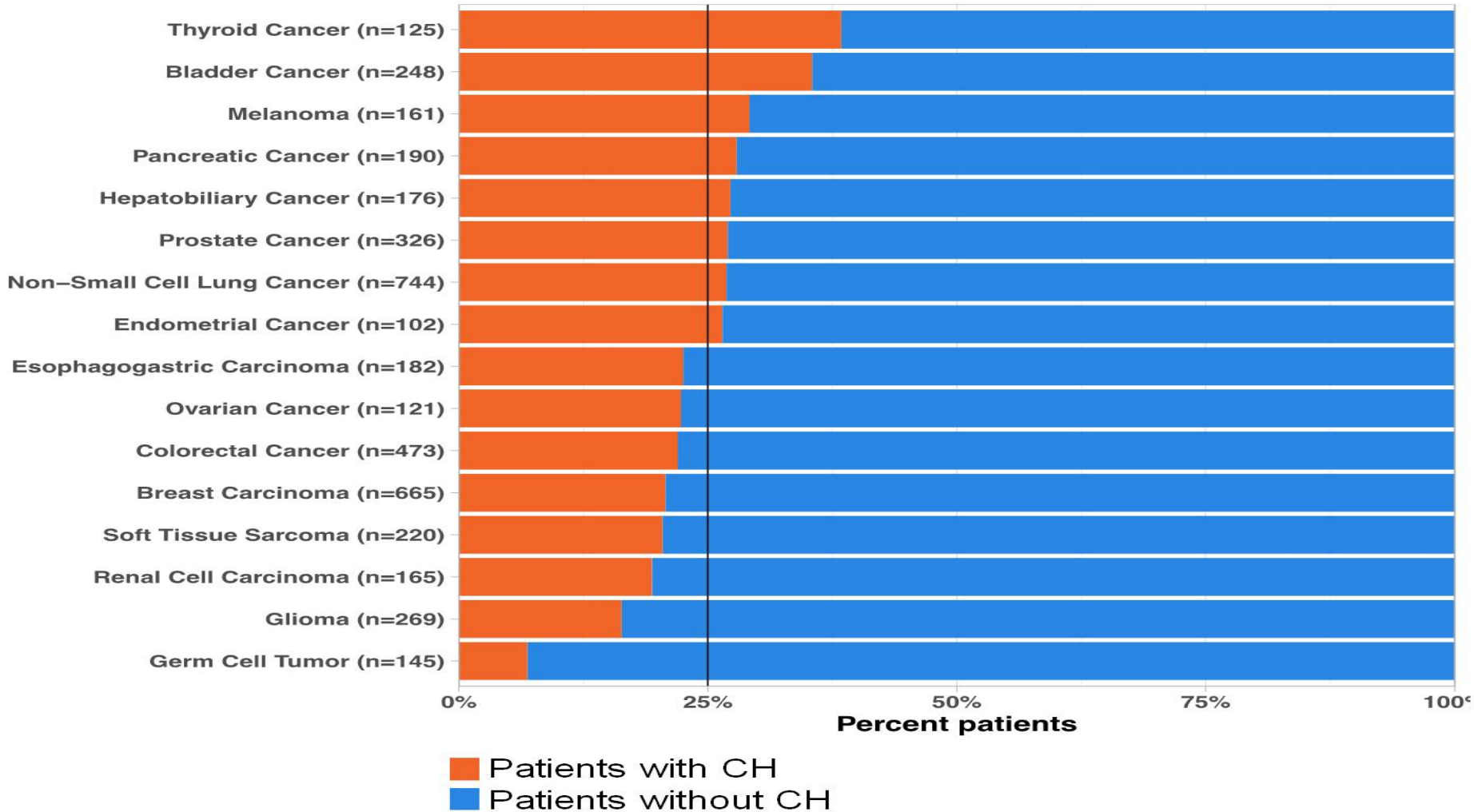
**NOT INFERIOR TO TUMOR POSITIVE , PLASMA POSITIVE
OR NEGATIVE**



**KEY CHALLENGE IS TO DISTINGUISH DOMINANT
MUTATIONS FROM PRECANCEROUS MUTATIONS
AND COOPERATING MUTATIONS**

ANOTHER PROBLEM : SOME MUTATIONS ARE CONFOUNDED BY THE FREQUENT FINDINGS OF CLONAL HEMATOPOIESIS OF UNDETERMINED SIGNIFICANCE :P53 IS COMMON IN THIS DISORDER AND EVEN RAS

Effects of Solid tumor type on CH



WHAT SHOULD WE HAVE TO FEEL COMFORTABLE AN ASSAY SHOWS CLINICAL UTILITY

- Robust numbers to accurately gauge specificity and sensitivity
- Correct controls for specificity
- Studies on performance and utility in the intended use population
- If discordant with a companion diagnostic , natural history of the discordant population determined to see which is truth

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CONCLUSIONS

- The technology is more advanced , maybe than the understanding of the biology
- How do we quantitate the new clone
- At this moment tissue biopsy should always be done , it is potentially dangerous given the frequency 10-20% of discordance in the relapse situation to use plasma and the possibility that in fact T-P+ patients may do poorly
- Tests are being marketed that have not had adequate specificity controls eg non EGFR mutant lung cancer specimens or using other cancer subtype specimens
- Trials should test clinical outcome in T-P+ patients separately if there is any discordant rate for that mutation