
A NEW VISION IN THE DIAGNOSTIC/PROGNOSIS OF CANCER USING QUANTITATIVE BIOLOGY

Prof. Antonio Ferrer-Montiel

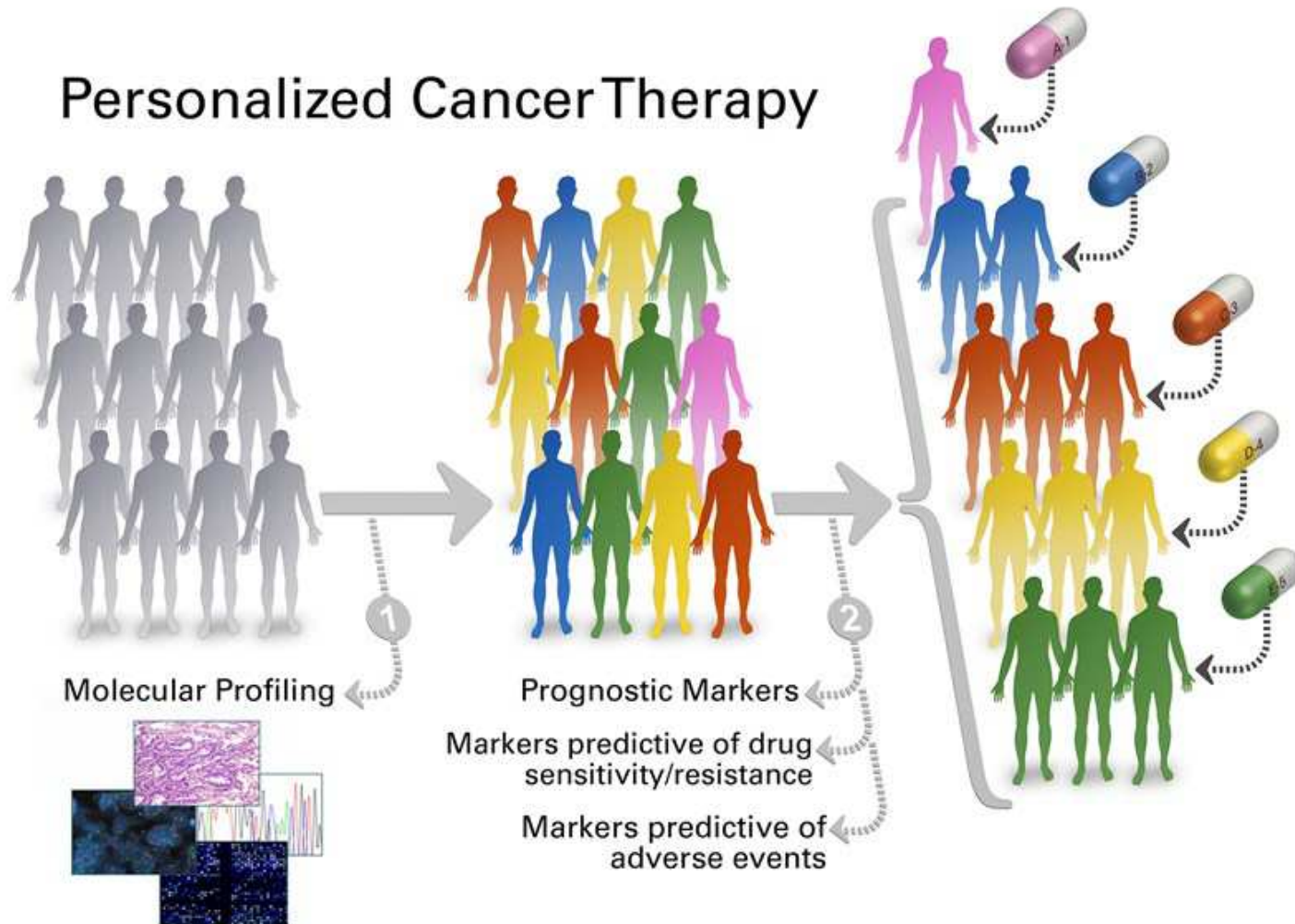
QUANTITATIVE BIOLOGY: A NECESSITY

- Objective measures
- Quantitative ranking
- Subjective bias elimination
- Data-based decision making



A need for proper biomarker validation

BIOMARKER NEED

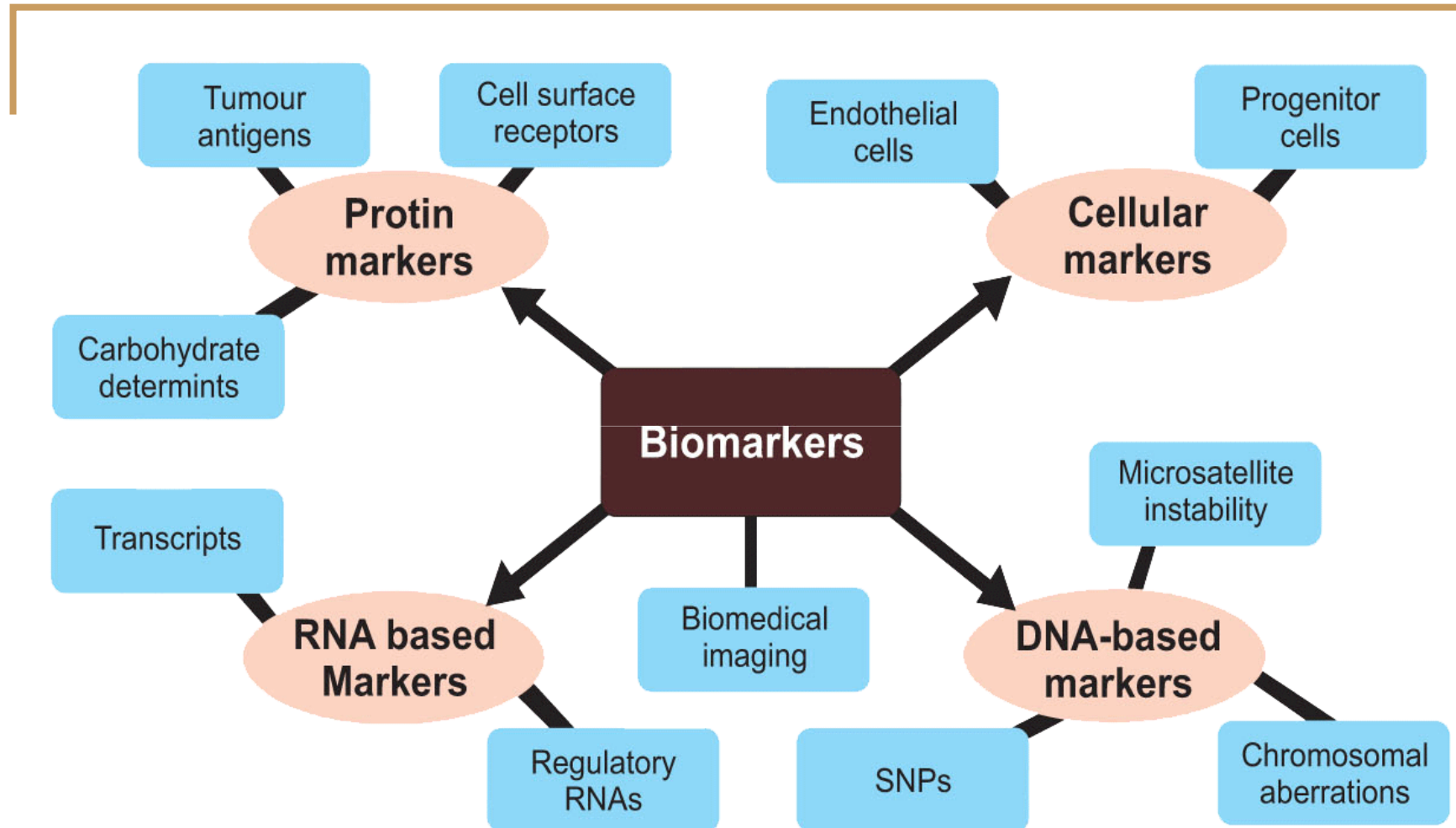


NEED FOR INNOVATION

- ❑ Increasing development of targeted therapies
- ❑ Limited precision in linking patient with therapy
- ❑ Poor linkage brings - a cost burden to society
- ❑ A missed opportunity for patients
- ❑ A quality of life burden to patients

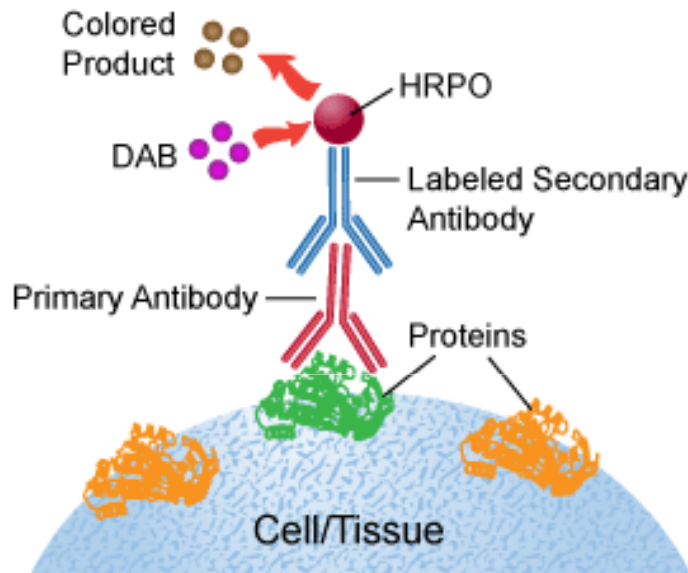


BIOMARKER EVOLUTION

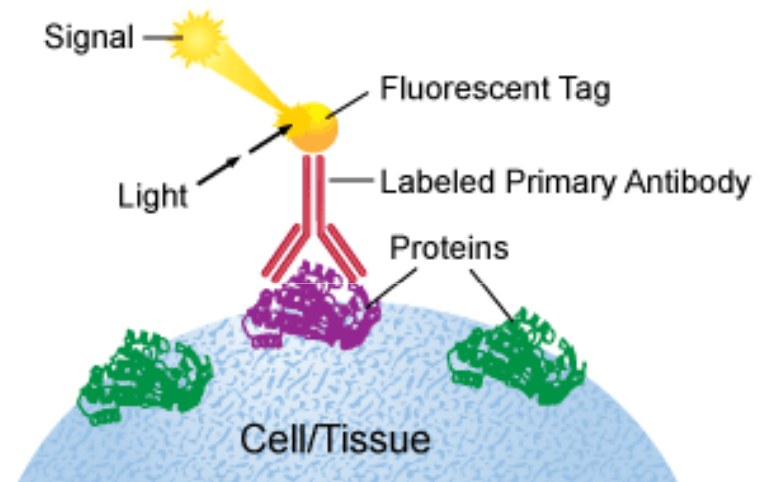


PROTEIN-BASED BIOMARKERS

Indirect Immunohistochemistry



Immunofluorescence



- Good specificity
- Cost effective
- Technically affordable
- Portable

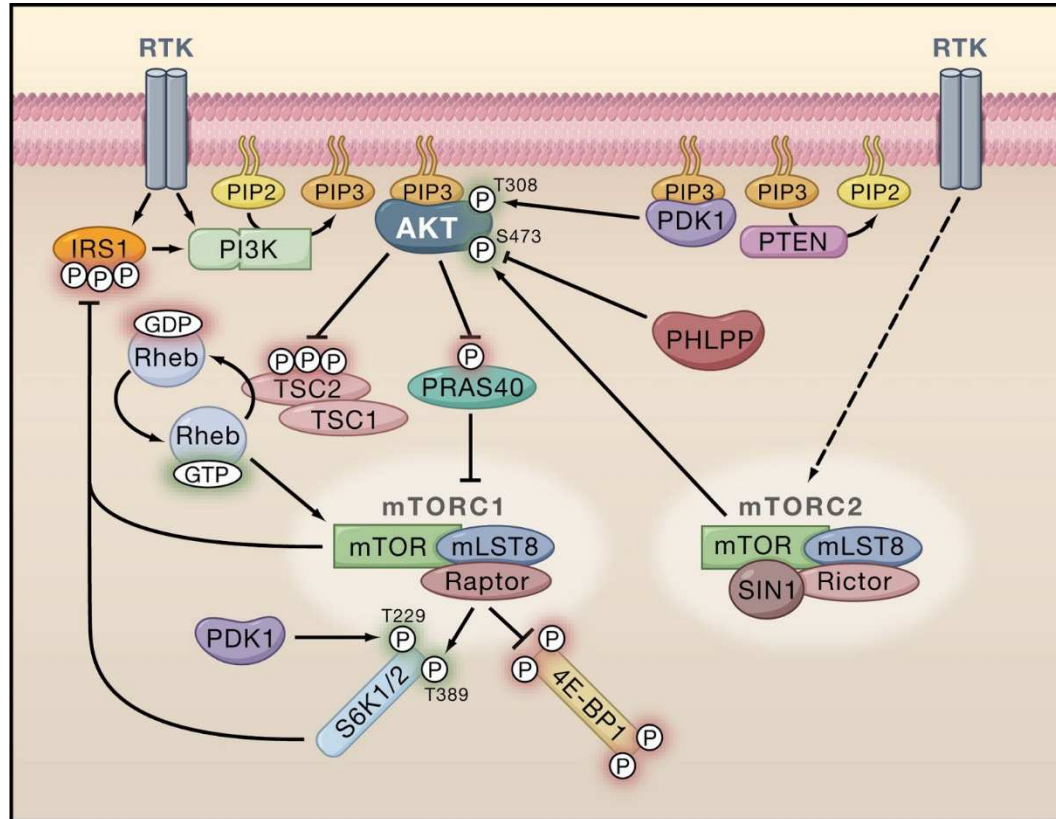
- One-site: only determines amount of biomarker
- No information on biomarker activity
- Limited sensitivity
- Poor quantification; qualitative measurement
- Poor prognosis with patient evolution

FUNCTIONAL PROTEOMICS

Biomarker activity may have higher prognosis than protein level

A case in point:

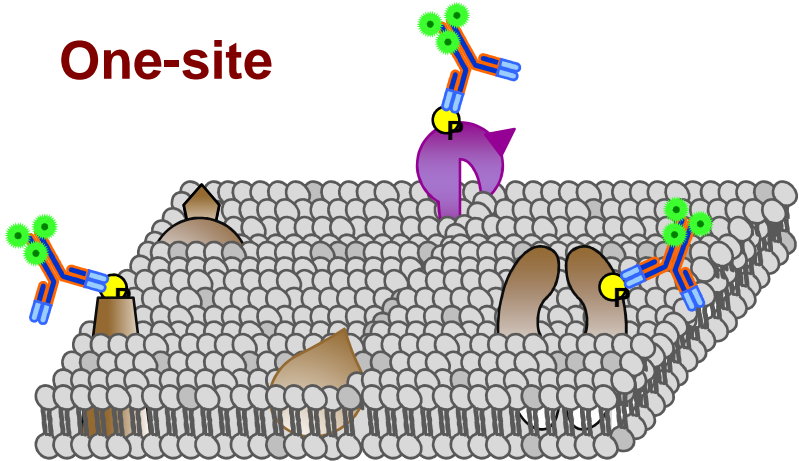
Akt/PKB phosphorylation



The need of measuring also biomarker activity: a two-side assay

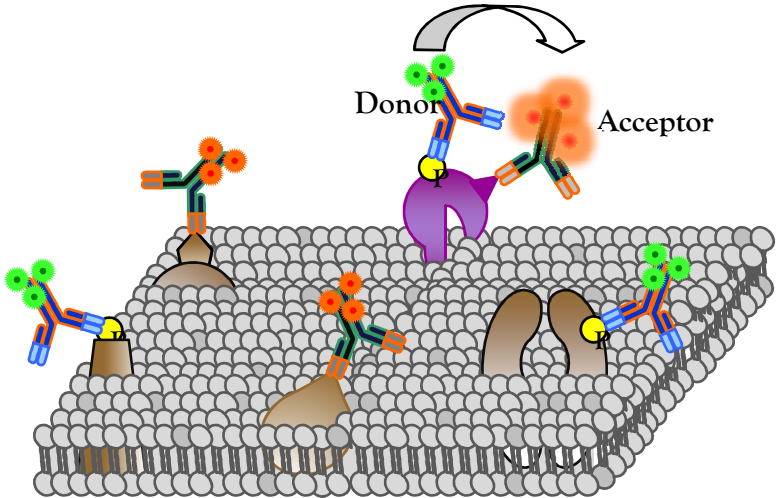
DESIGN OF A TWO-SITE ASSAY: FRET

One-site



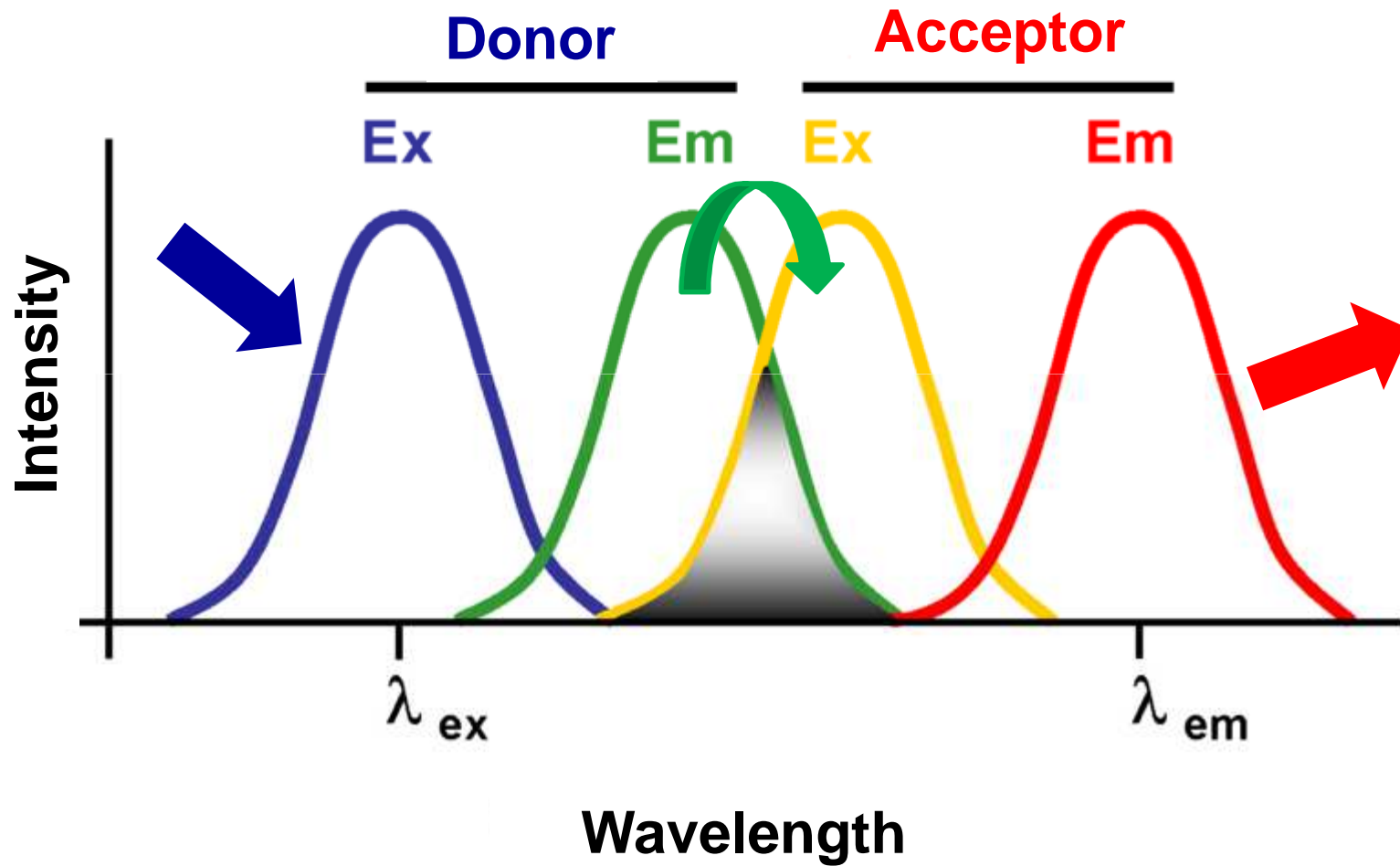
Two-site

FRET

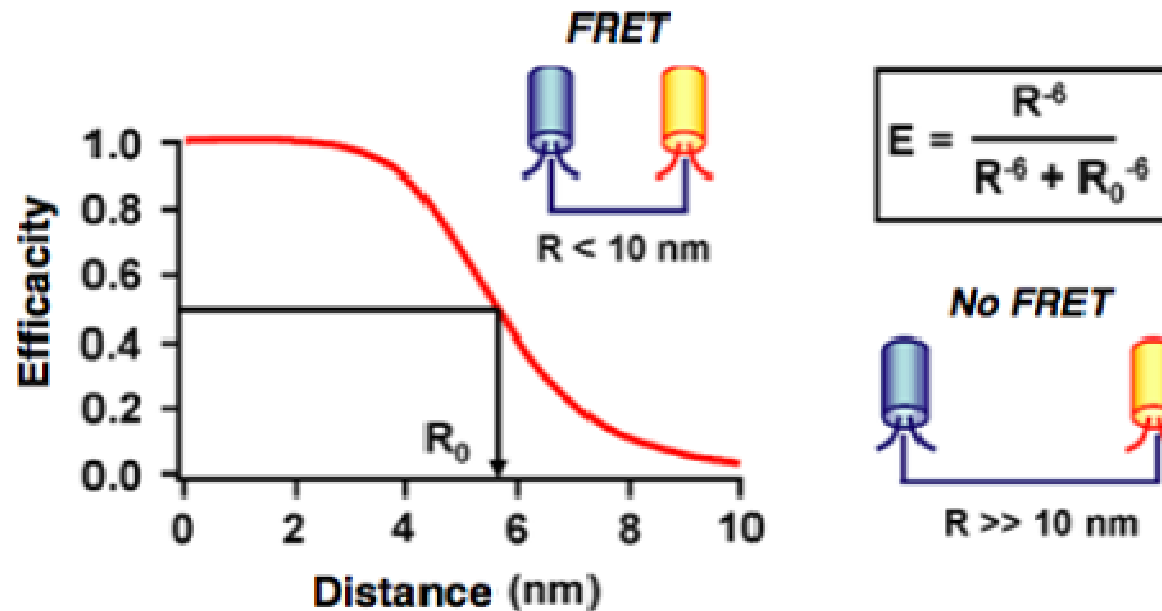


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FLUORESCENCE RESONANCE ENERGY TRANSFER



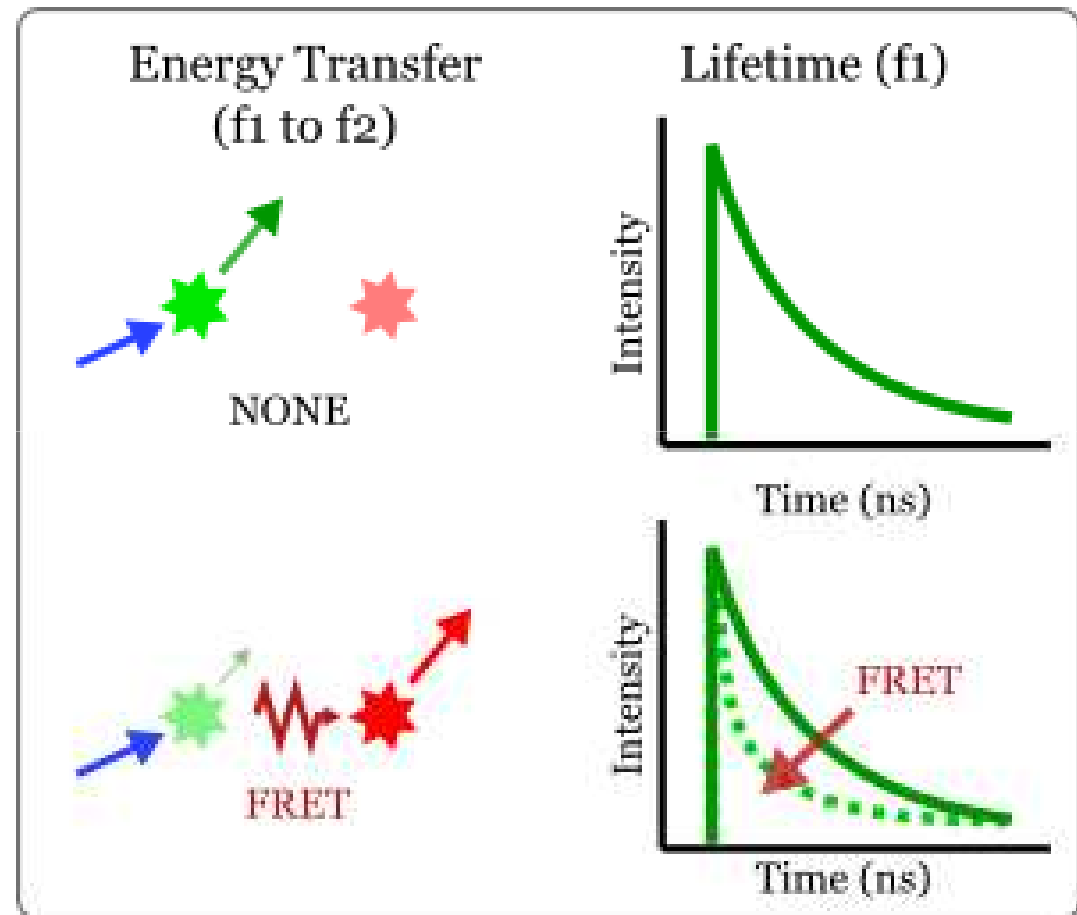
FRET EFFICIENCY CRITICALLY DEPENDS ON DISTANCE



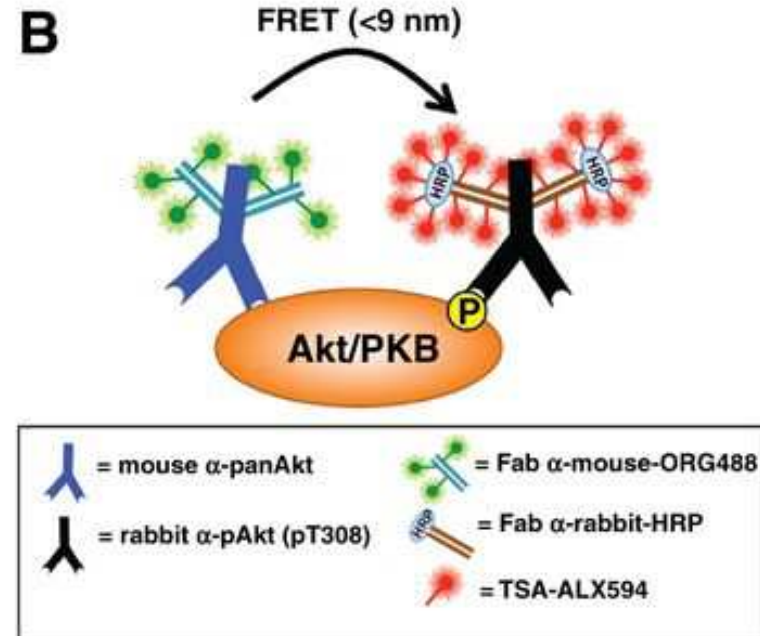
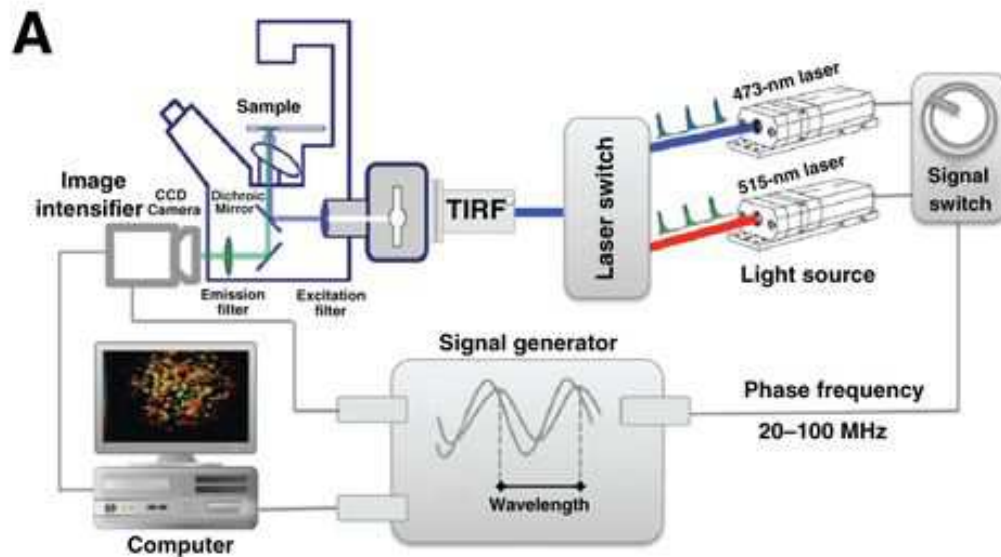
- Efficiency depends on concentration of donor and acceptor
- Efficiency depends on excitation intensity.

FLUORESCENCE LIFETIME (FLIM)

- Affected by Environmental factors
- Affected by FRET
- Independent of concentration
- Independent of excitation intensity

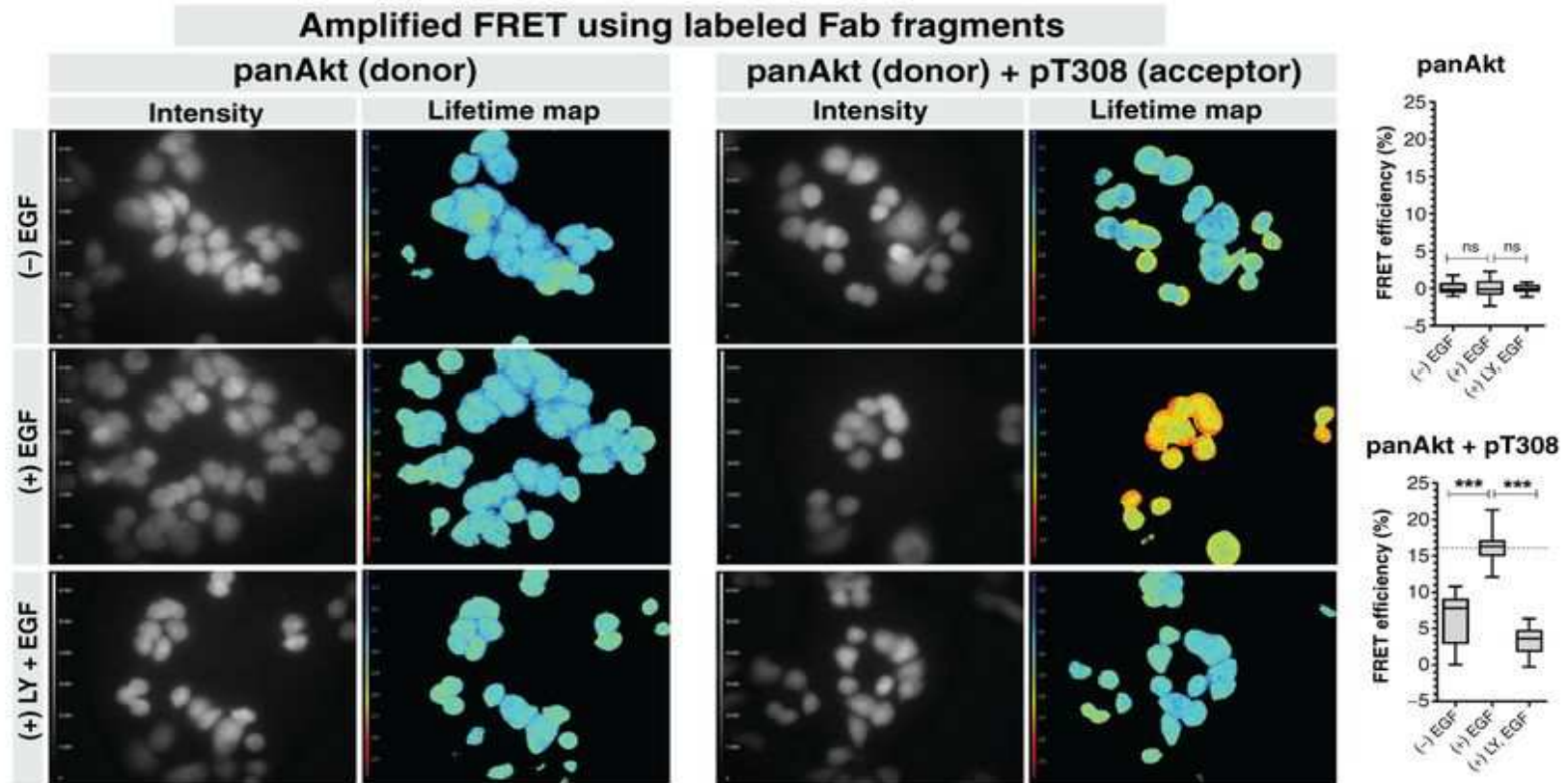


AMPLIFIED FRET-FLIM

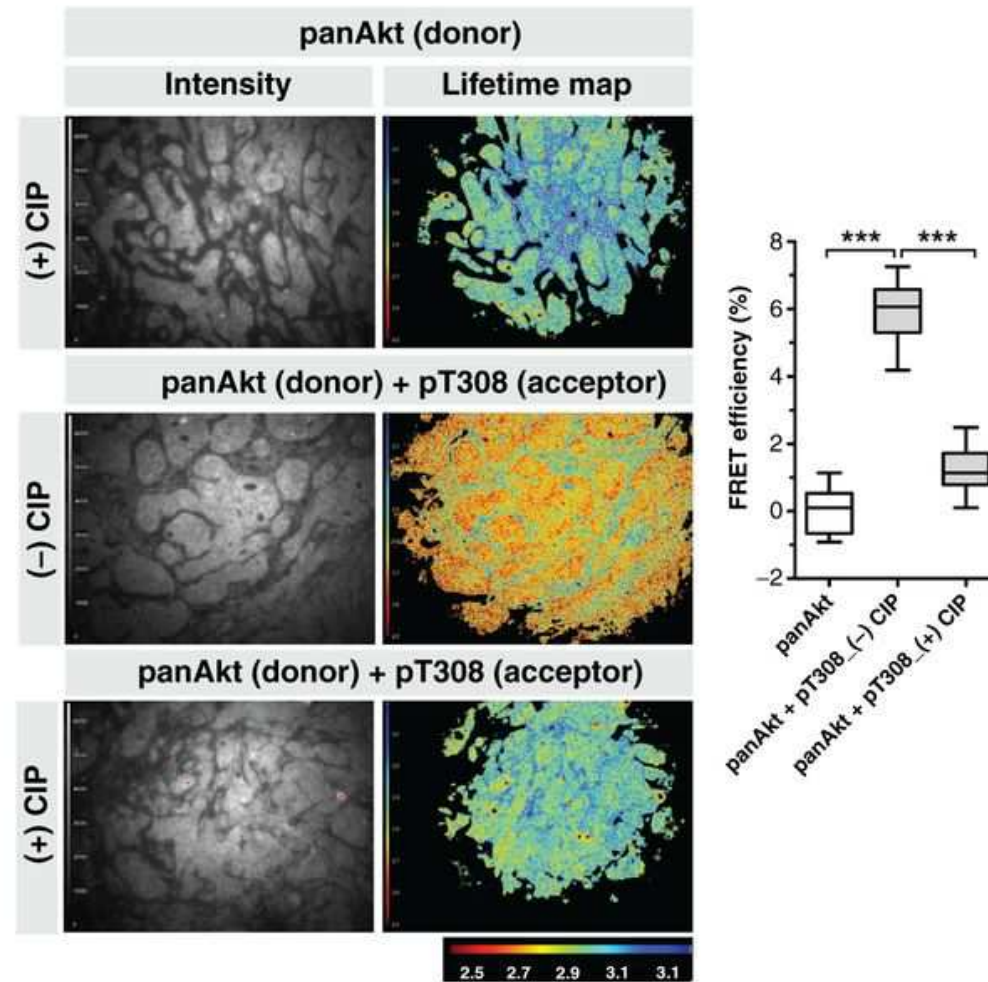


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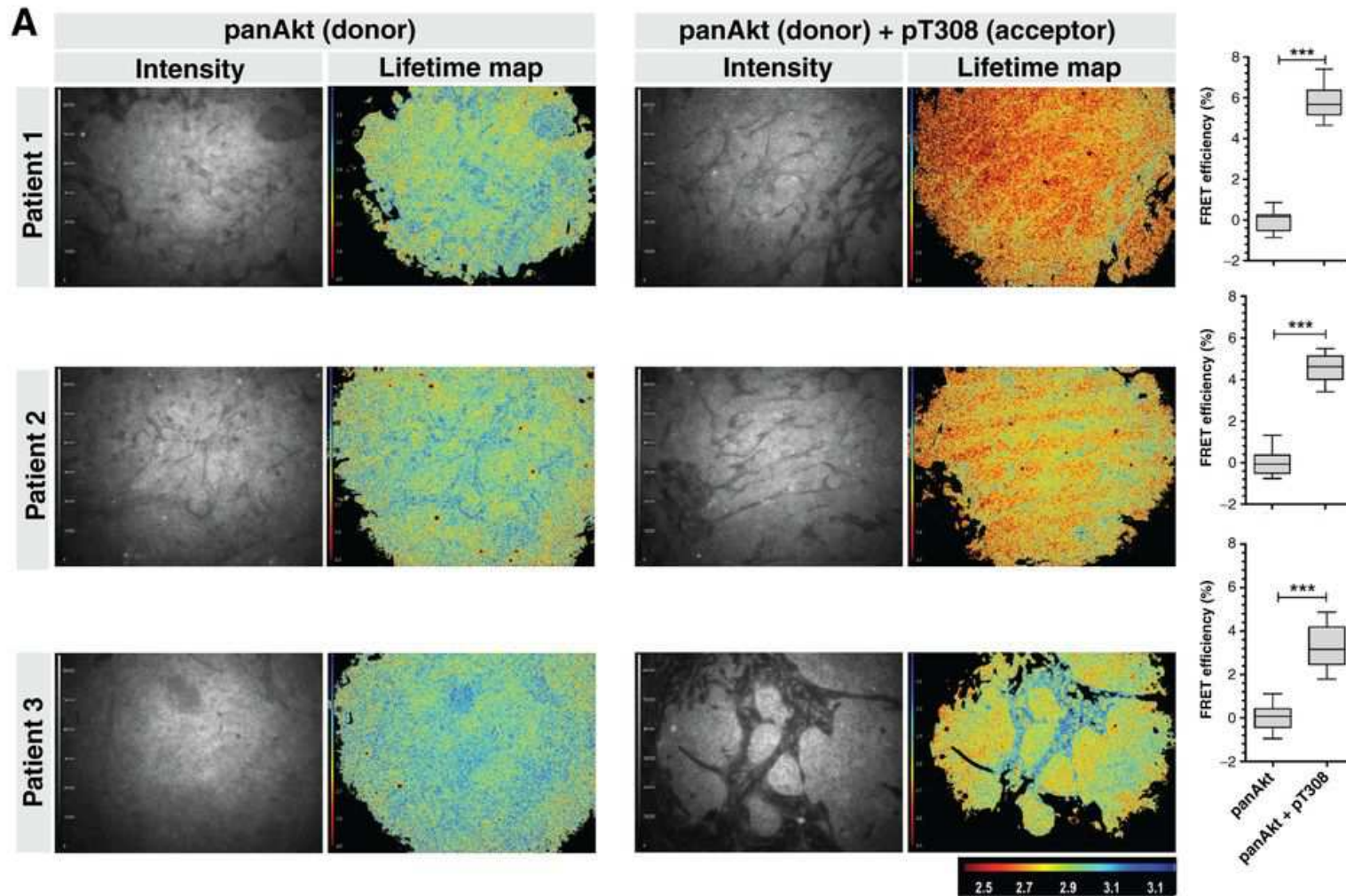
AMPLIFIED FRET-FLIM IN TUMOUR CELLS



AMPLIFIED FRET-FLIM DEPENDS ON AKT PHOSPHORYLATION

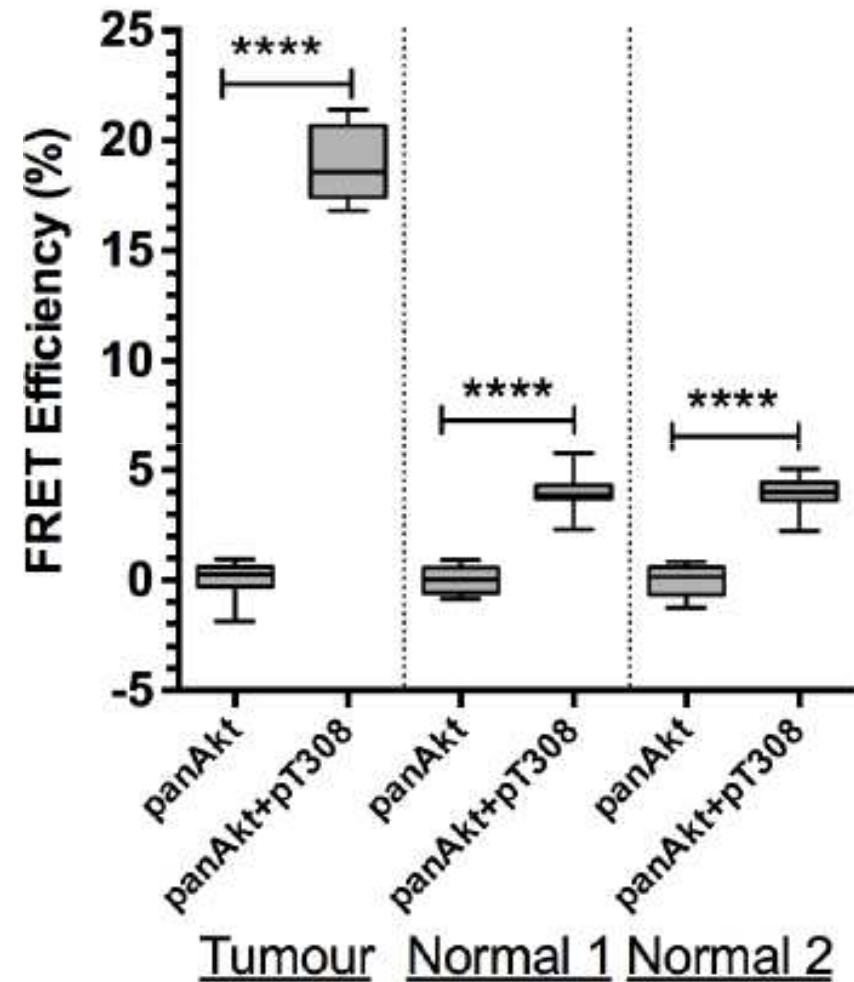


AMPLIFIED FRET-FLIM IN PATIENT TUMOURS



HIGH DYNAMIC RANGE

- E_f has a dynamic range from 5 to 30%
- E_f for normal breast tissue is 2.3% to 5.7 % (median average FRET efficiency of 4%)



HIGH THROUGHPUT TECHNOLOGY

Microarray Panel Display

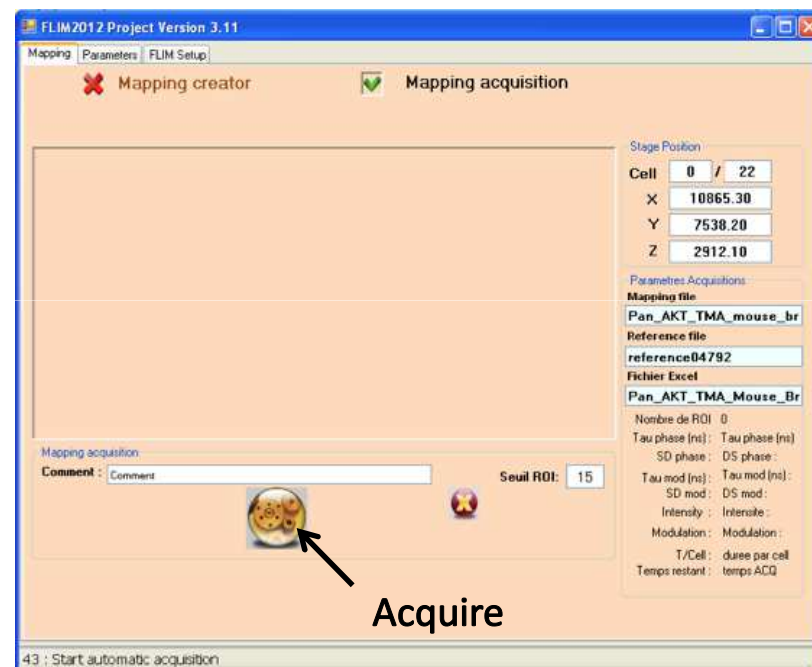
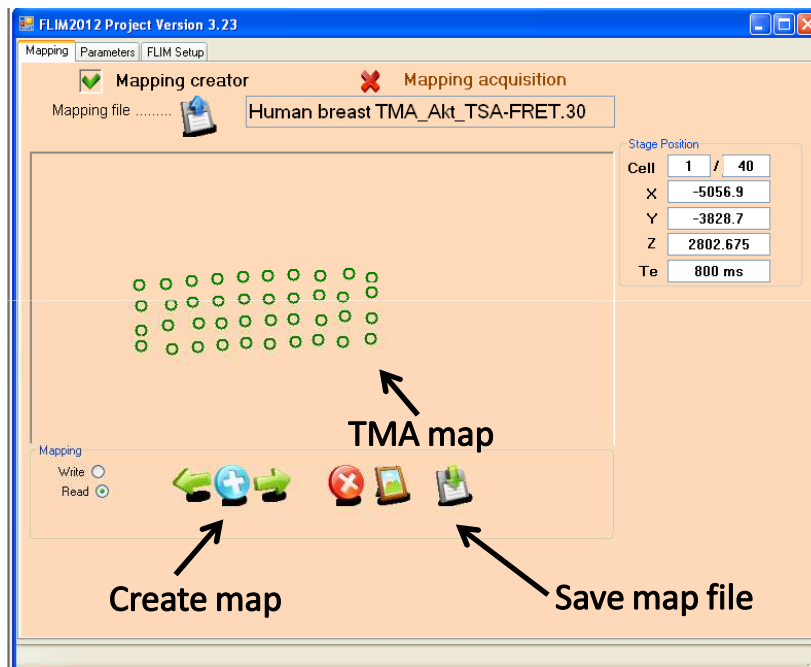
	1	2	3	4	5	6	7	8	9	10	11	12
A	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
B	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
C	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
D	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
E	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
F	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
G	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre
H	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre	bre

Legend: bre - breast

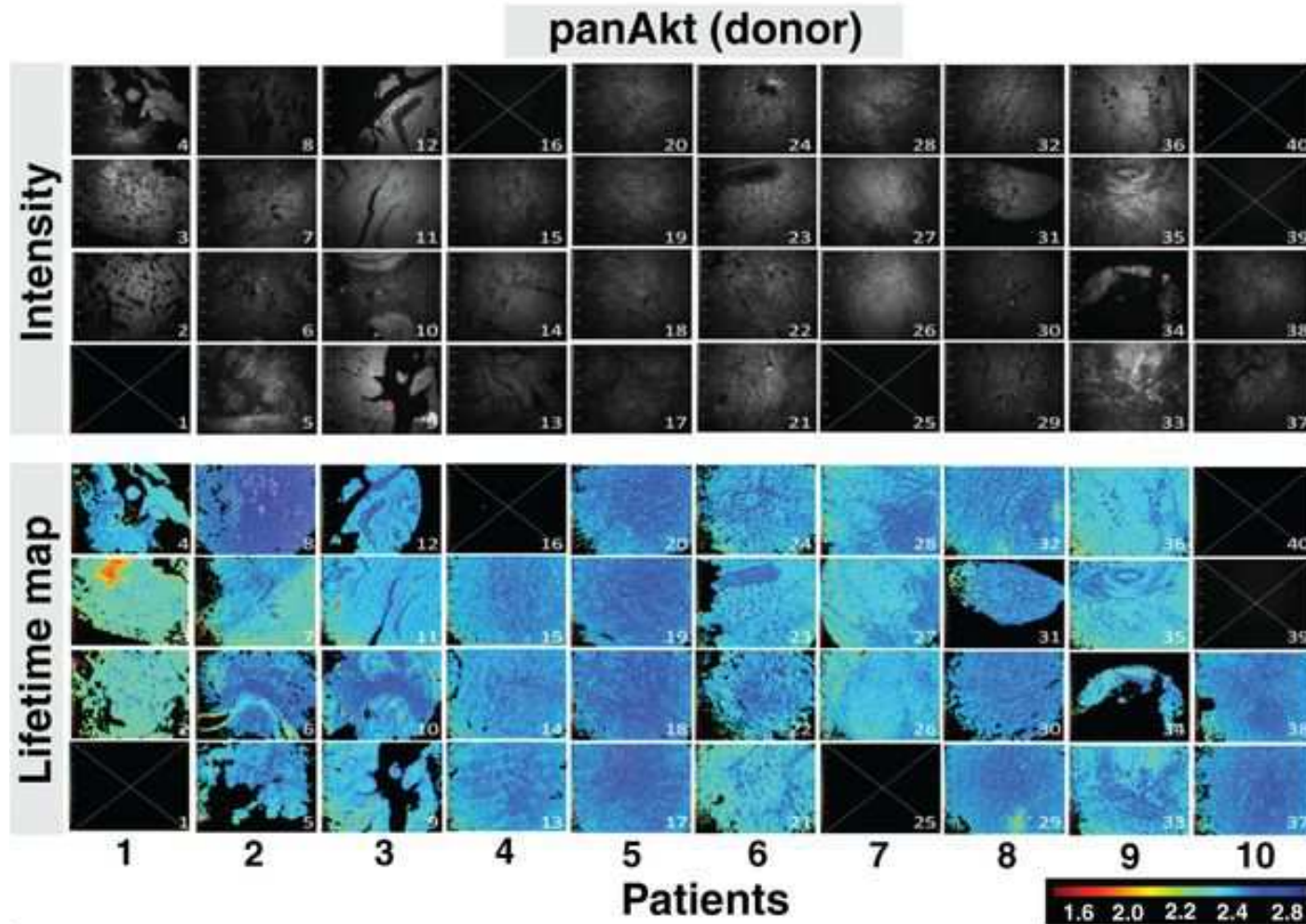
● - Benign tumor, ● - Hyperplasia, ● - In Situ, ● - Malignant tumor, ● - Normal tissue, ○ -



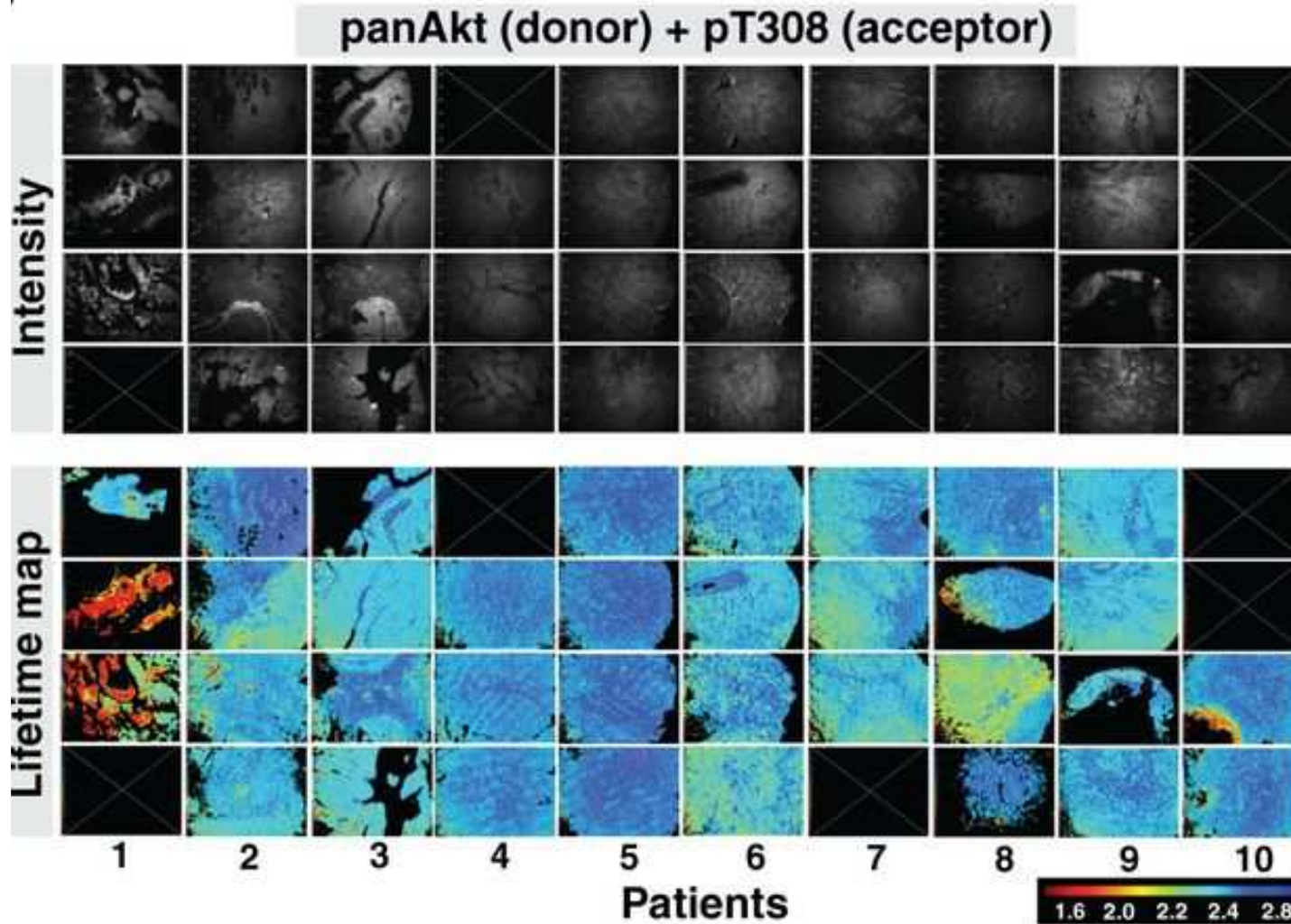
AUTOMATED MAPPING AND ACQUISITION



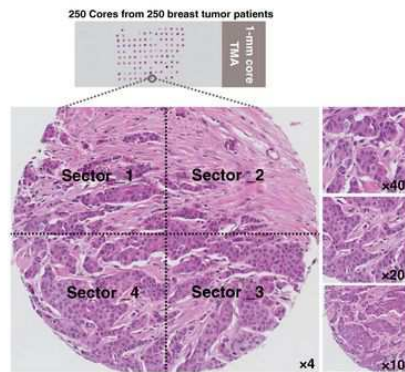
AMPLIFIED FRET-FLIM IN A COHORT OF PATIENT TUMOURS



AMPLIFIED FRET-FLIM IN A COHORT OF PATIENT TUMOURS

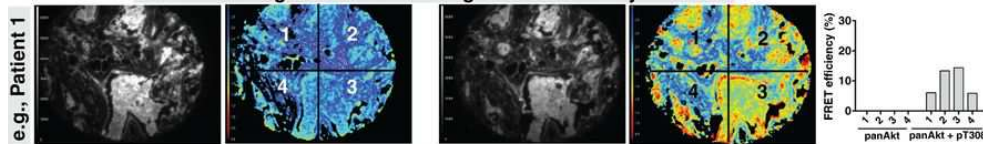


AMPLIFIED FRET-FLIM IN TUMOUR HETEROGENEITY

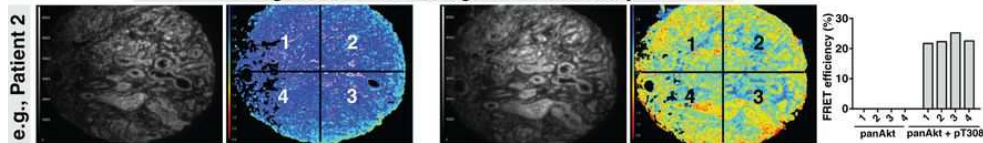


panAkt (donor)		panAkt (donor) + pT308 (acceptor)	
Intensity	Lifetime map	Intensity	Lifetime map

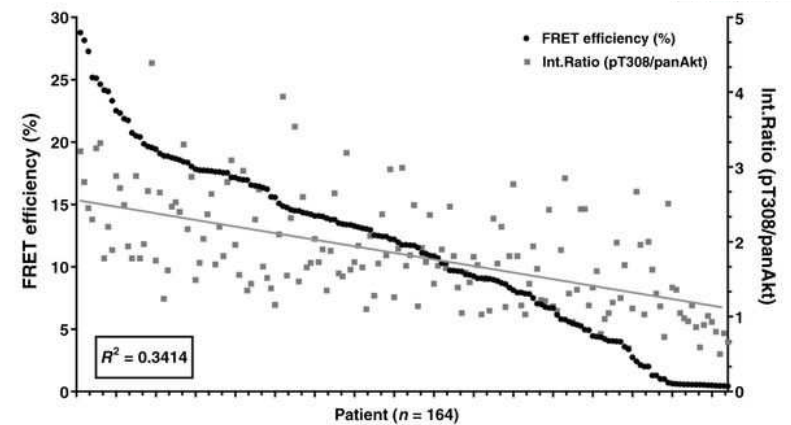
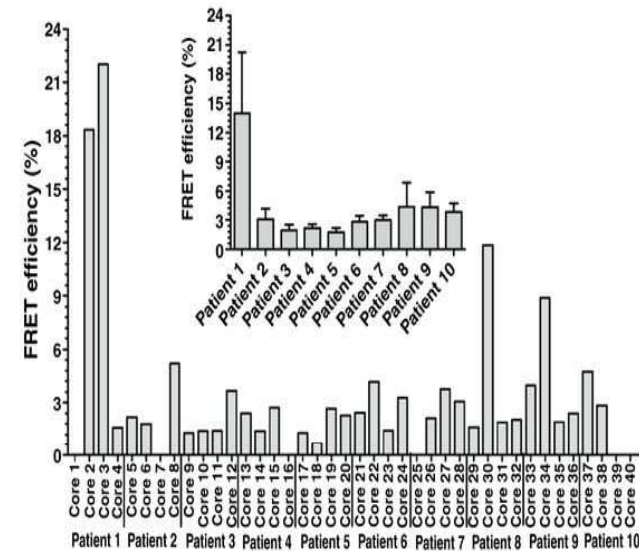
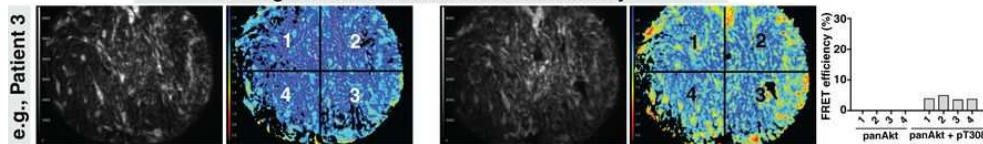
Heterogeneous ROI with high FRET efficiency



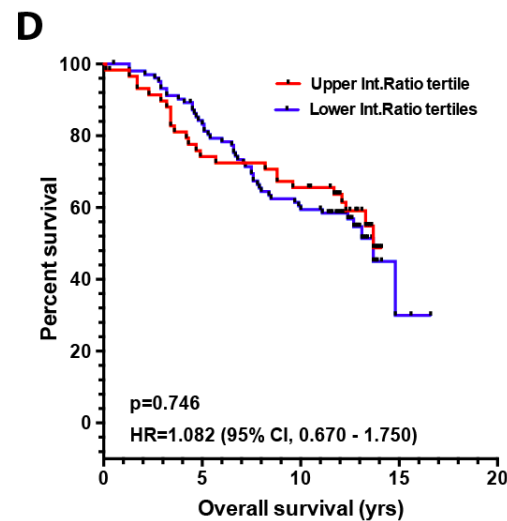
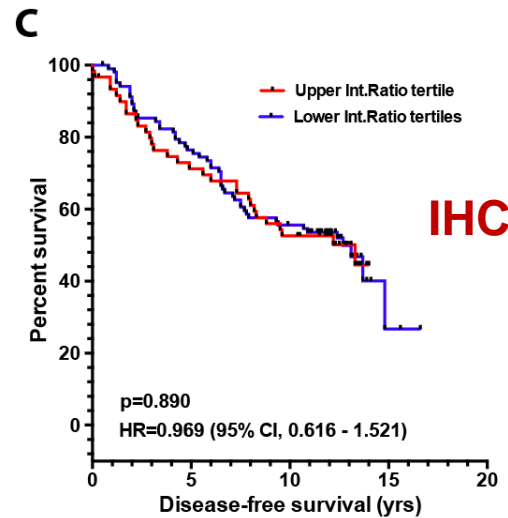
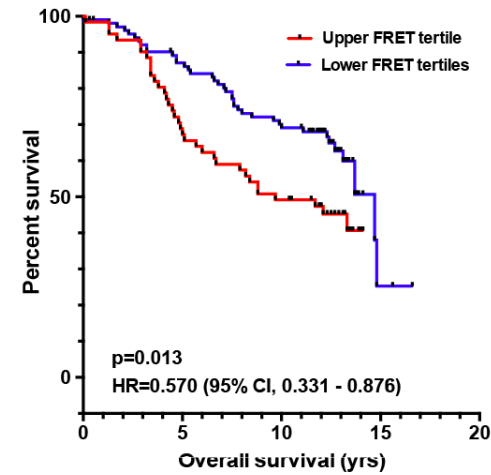
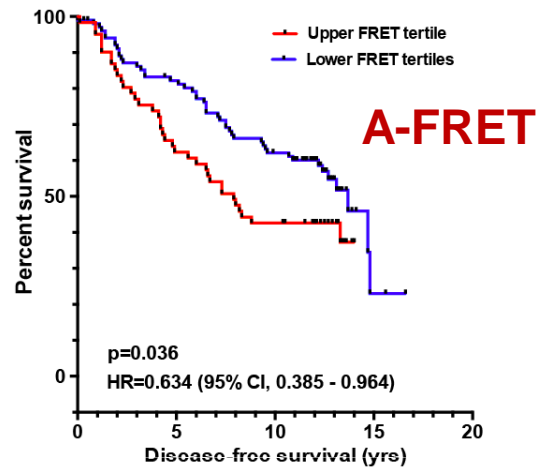
Homogeneous ROI with high FRET efficiency



Homogeneous ROI with low FRET efficiency

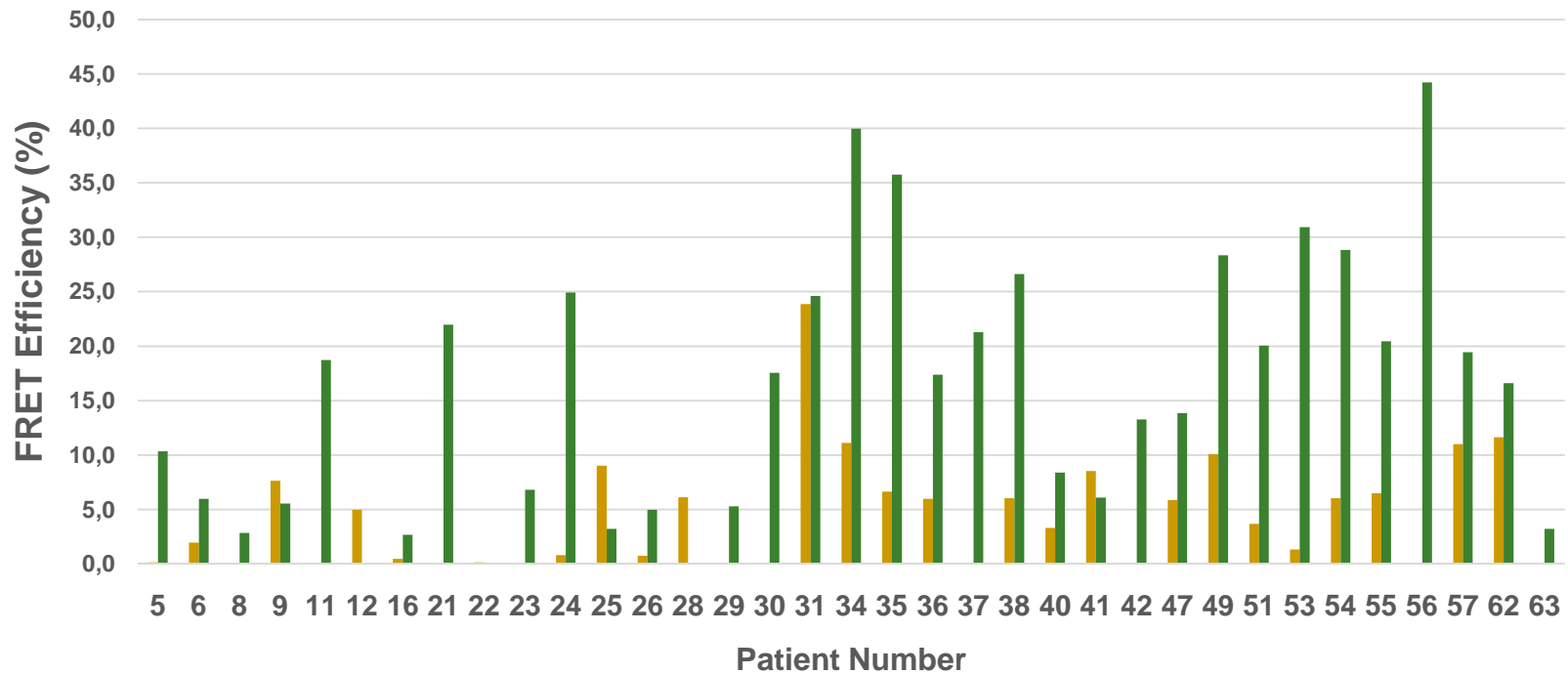


AKT ACTIVATION IS INDICATIVE OF POOR PROGNOSIS



CLEAR CELL RENAL CARCINOMA

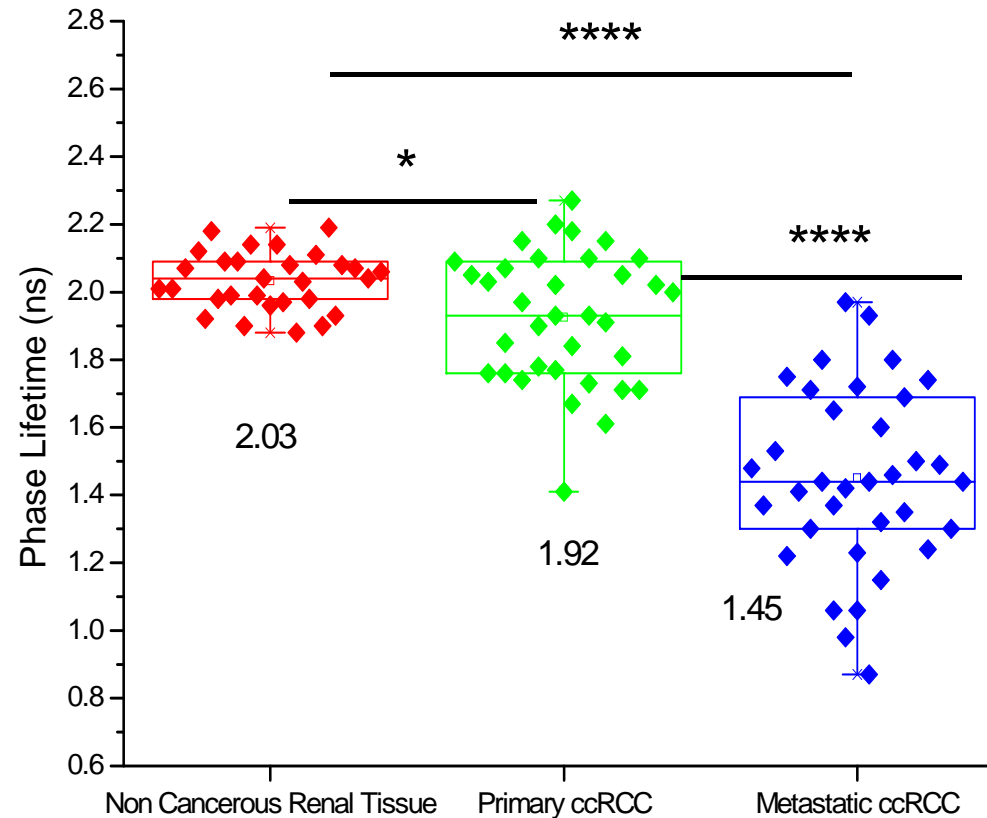
Primary vs Metastatic FRET Efficiency per Patient



■ Fret Efficiency Primary (%)

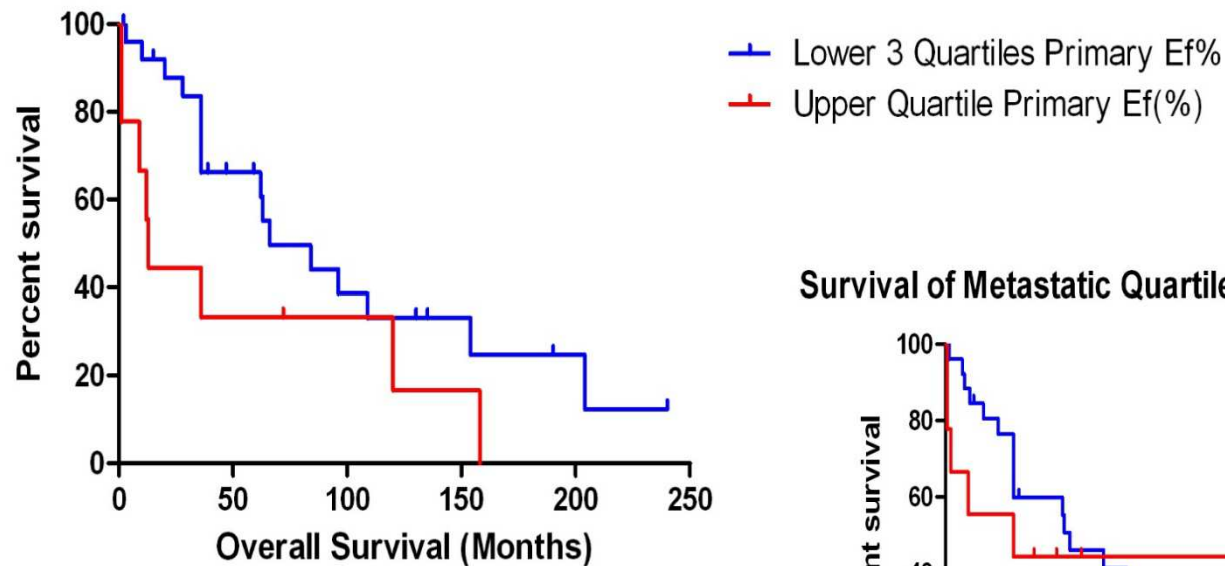
■ Fret Efficiency Metastatic(%)

FRET LIFETIMES FOR AKT/PKB IN CLEAR CELL RENAL CARCINOMA

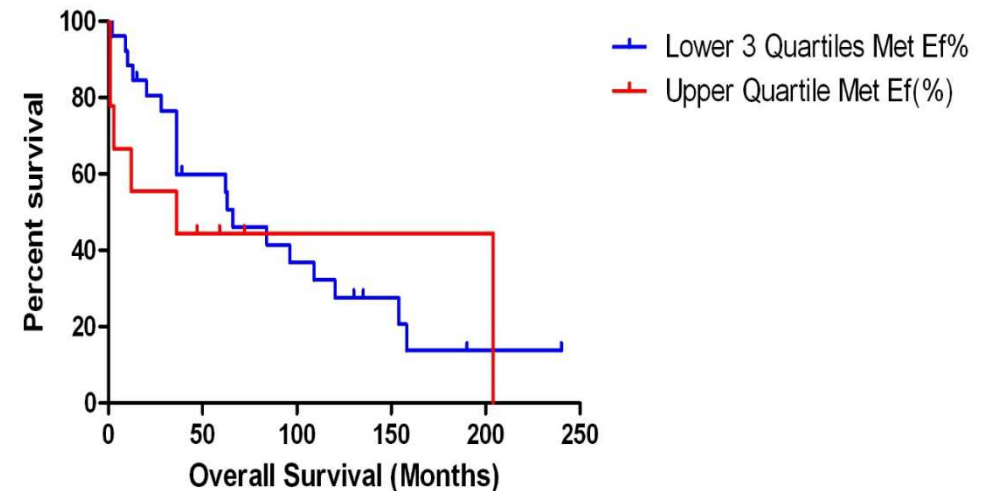


SURVIVAL CURVES FOR PRIMARY AND METASTATIC TUMOURS

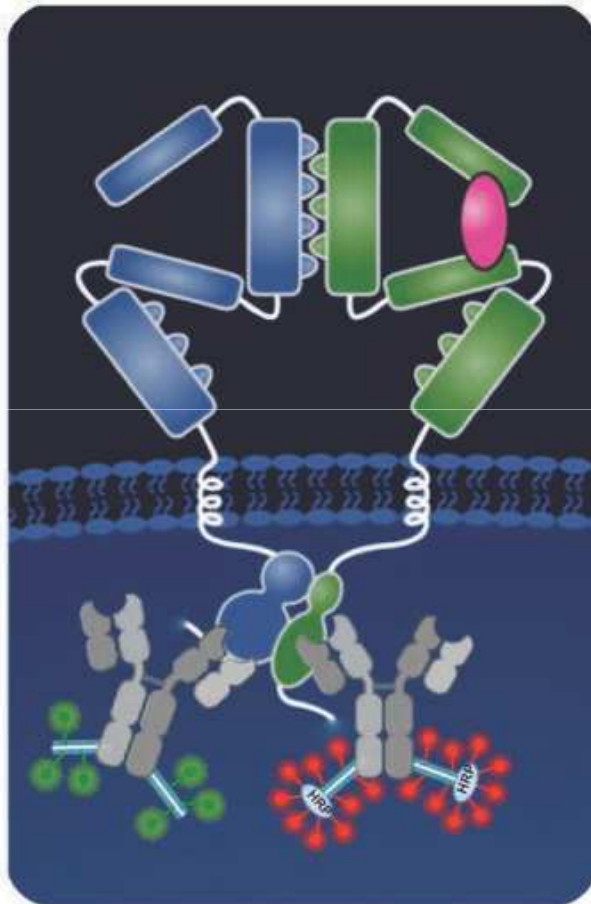
Survival of Primary Quartiles: Survival proportions



Survival of Metastatic Quartiles: Survival proportions



DIMERISATION OF HER2/HER3 BY A-FRET



HER2 = Donor
HER3 = Acceptor

HER2 (rabbit)	HER3 (mouse)
(surrounding Tyr aa1248)	(aa1175-1275)

A-FRET-FLIM COMPARISON

	IHC	PLA	Amplified-FRET
Sensitivity	+	++	++++
Selectivity	+	+++	+++
Dynamic Range	+	++	++++
Protein-Protein interaction	-	++	++++
Modified Proteins	+	+++	++++

ADVANTAGES OF AMPLIFIED FRET-FLIM

- ❑ Significantly **higher signal/noise ratio** – critical in use of FRET/FLIM on tissue sections.
- ❑ Ability to generate **higher spatial resolution** – crucial in investigating tumour heterogeneity
- ❑ **Higher dynamic range** – enabling accurate measurements.
- ❑ Ability to **quantify modified proteins** (e.g. p-AKT) as a fraction of total protein.
- ❑ Ability to analyse **protein-protein interaction** (e.g. AKT-PDK1, Her2/Her3).

AMPLIFIED FRET-FLIM TECHNOLOGY HAS BEEN PROTECTED

- ❑ Current: **GB 304352.6**- Patent filed in March 2013/**Now PCT published**-IP with Francis Crick Institute-London
- ❑ Inventors: Banafshé Larijani, Peter Parker and Selvaraju Veeriah

CONCLUSIONS

- ❑ Amplified FRET-FLIM a versatile, sensitive technology to measure biomarker expression and function
- ❑ Amplified FRET-FLIM correlates with cancer prognosis
- ❑ Amplified FRET-FLIM addresses tumour and patient heterogeneity
- ❑ Amplified FRET-FLIM is high throughput and automated
- ❑ Amplified FRET-FLIM is quantifiable and highly sensitive
- ❑ Amplified FRET-FLIM is a novel technology for functional proteomics
- ❑ Amplified FRET-FLIM outperforms IHC and genomic approaches
- ❑ Amplified FRET-FLIM is a companion diagnostic tool in cancer
- ❑ FASTBASE SOLUTIONS aims to translate this technology to patients

FASTBASE SOLUTIONS

Decision with precision

<http://www.fastbasesolutions.com/>



*A New Vision of Cancer
Diagnostics and prognosis*

Crowdfunding (from 5€)

<https://fr.ulule.com/fastbase-solutions/>

THE PROMOTERS

<ul style="list-style-type: none">• Prof. Banafshé Larijani is the head of Cell Biophysics Laboratory and Ikerbasque Research Professor. Banafshé works at the cutting edge of cross-disciplinary cancer biology and biophysics.	
<ul style="list-style-type: none">• Mr. Pierre Leboucher is Director of Development PRISME Platform at Brain & Spine Institute (Paris) with expertise at the interface of biomedicine, electronics and informatics.	
<ul style="list-style-type: none">• Prof. Peter J Parker Head of the Protein Phosphorylation Laboratory, at The Crick Institute and Division of Cancer Studies King's College (London). Peter is a pioneer in signalling pathways and cancer, and is co-founder of three companies.	
<ul style="list-style-type: none">• Prof. Antonio Ferrer-Montiel Acting R&D consultant of FASTBASE (Director of Instituto de Biología Molecular y Celular, Universitas Miguel Hernández, Alicante, Spain). Antonio is a molecular biologist that works at the front-line of membrane signalling and translational biophysics and skin biology.	

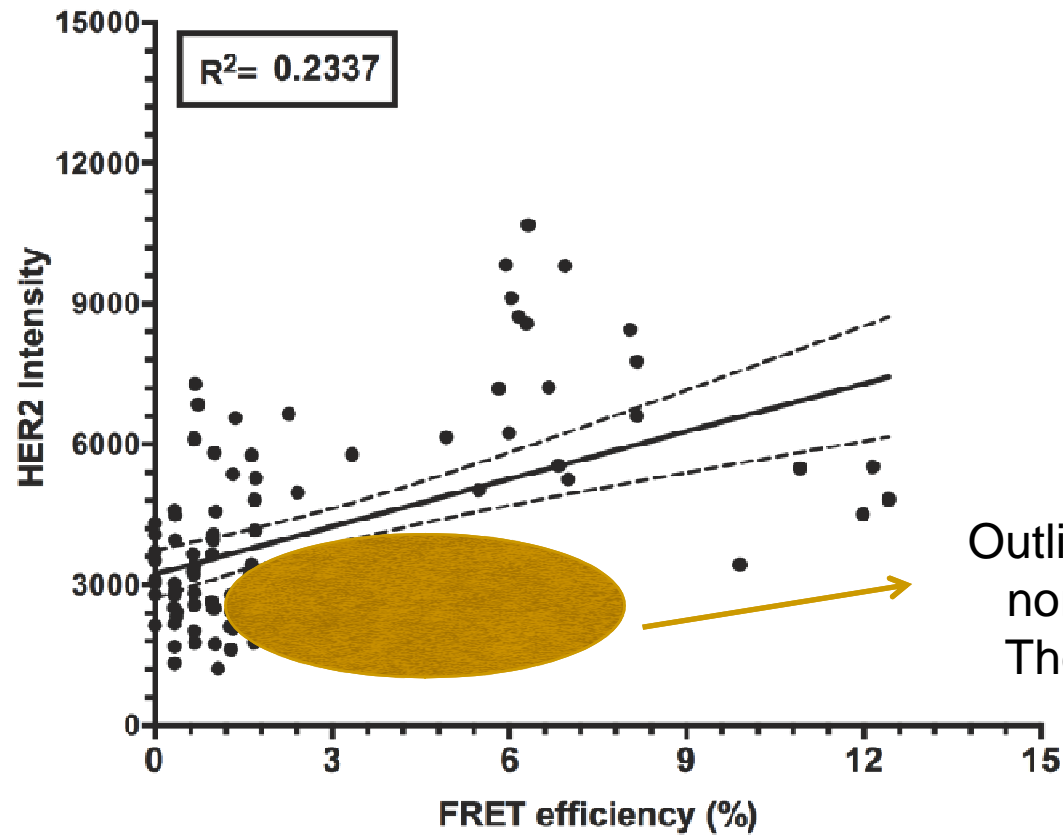
TUMOUR COHORT

Median age	57.0
Median FRET efficiency	11.8
Median intensity ratio (pT308/panAkt)	1.7
Grade	%
Grade 1	17.1
Grade 2	31.7
Grade 3	42.7
Unknown	7.9
ER	%
ER ⁺	76.2
ER ⁻	22.0
Unknown	1.8
PR	%
PR ⁺	55.5
PR ⁻	42.7
Unknown	1.8
HER2	%
HER2 ⁺	9.1
HER2 ⁻	37.2
Unknown	53.7

Adjuvant therapy	%
Adjuvant therapy (total)	86.0
Tamoxifen	62.8
CMF + tamoxifen	12.8
CMF	8.5
None	5.5
EFC	3.7
Ov Abl + tamoxifen	2.4
FEC + tamoxifen	2.4
Ov abl	1.2
APD	0.6
Surgery	%
Mastectomy	44.5
Conservation	55.5
Radiation therapy	%
Yes	55.5
No	44.

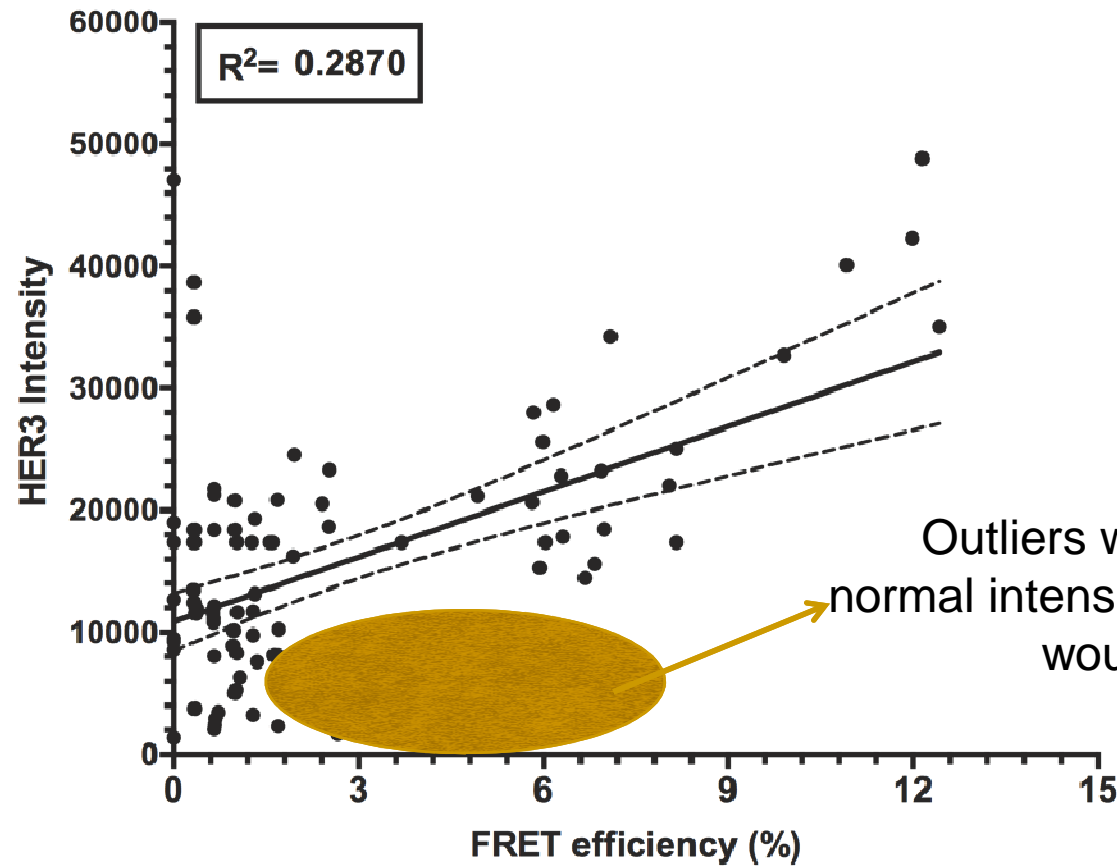
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HER2 INTENSITY WITH E_F



Outliers who would be missed by normal intensity measurements
Therefore would not be treated !

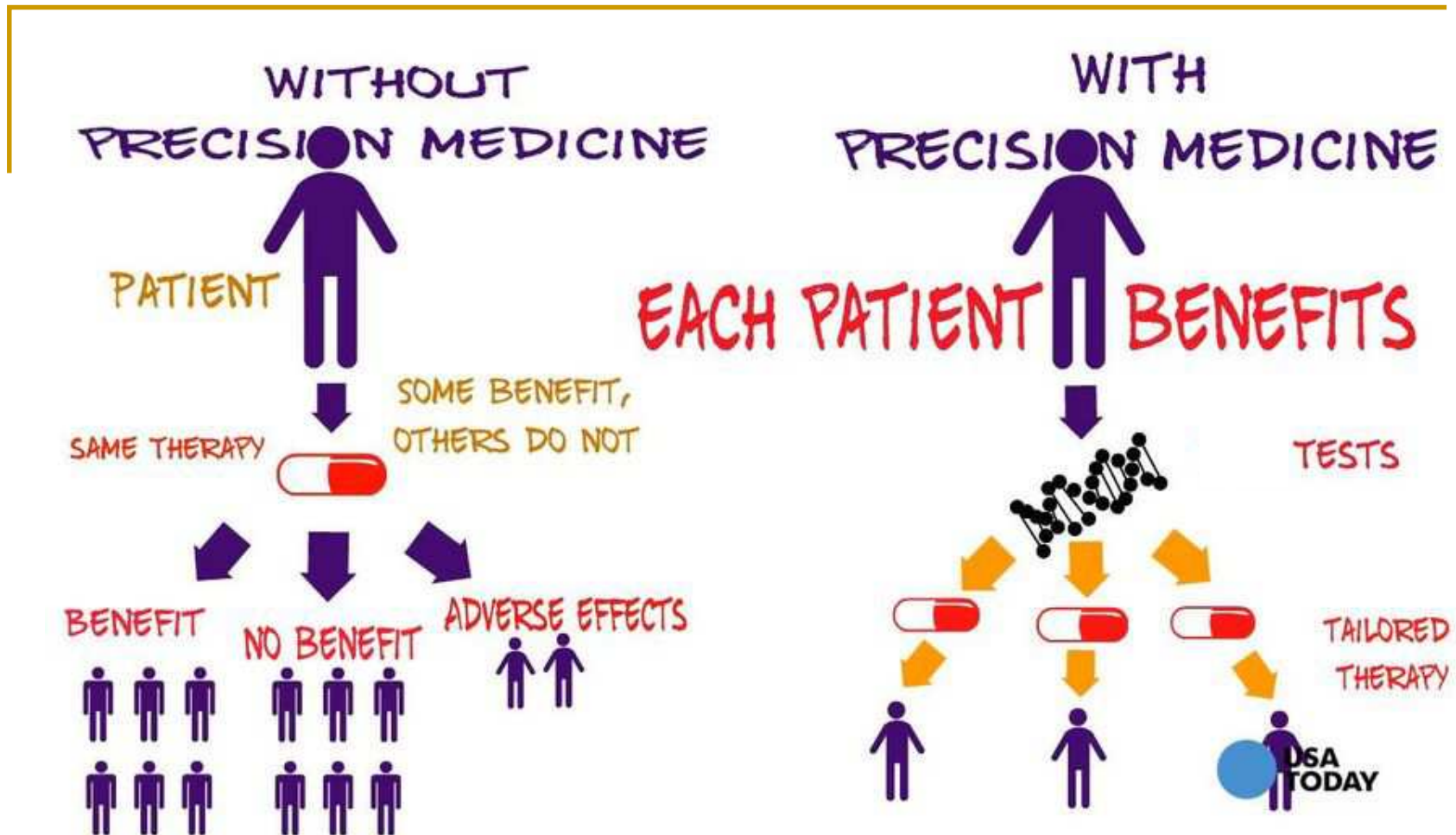
HER3 INTENSITY WITH E_f



Outliers who would be missed by normal intensity measurements Therefore would not be treated !

Banafshé Larijani PhD

FASTBASE SOLUTIONS



FASTBASE SOLUTIONS

PROBLEM

PATIENT STRATIFICATION FOR TREATMENT

Targeted therapies are directed at disease triggers

SOLUTION

A fast and efficient platform technology to measure drug target function and prognostic biomarkers