



MOLECULAR MARKERS OF TRASTUZUMAB RESISTANCE IN HER-2 METASTATIC BREAST CANCER PATIENTS

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University of Pisa - Italy

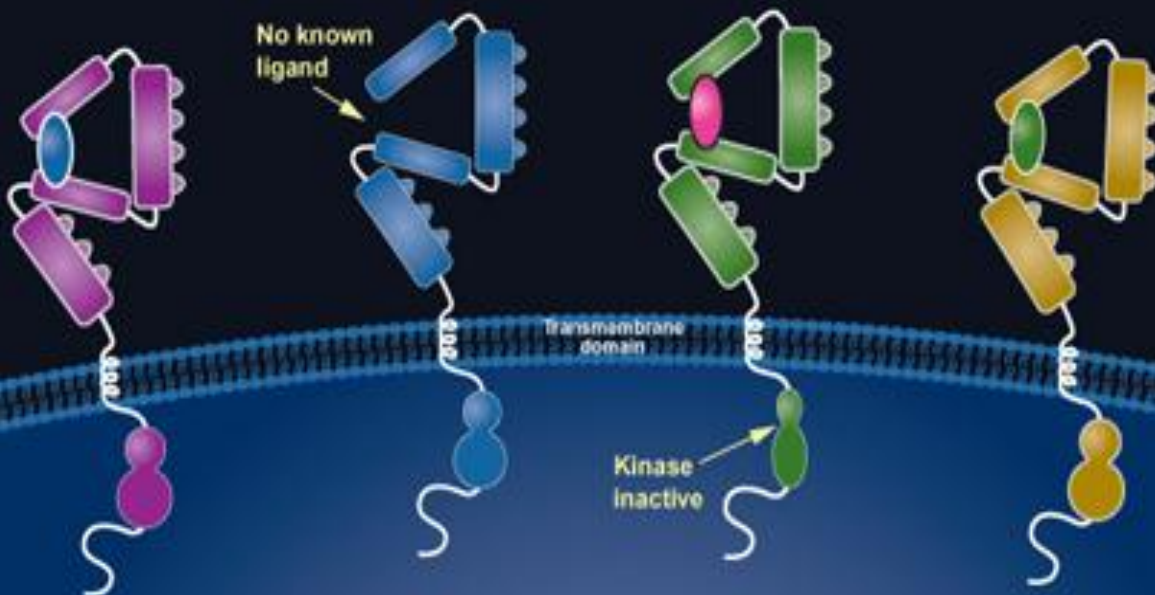
Extracellular ligand-binding domain

EGFR

HER2

HER3

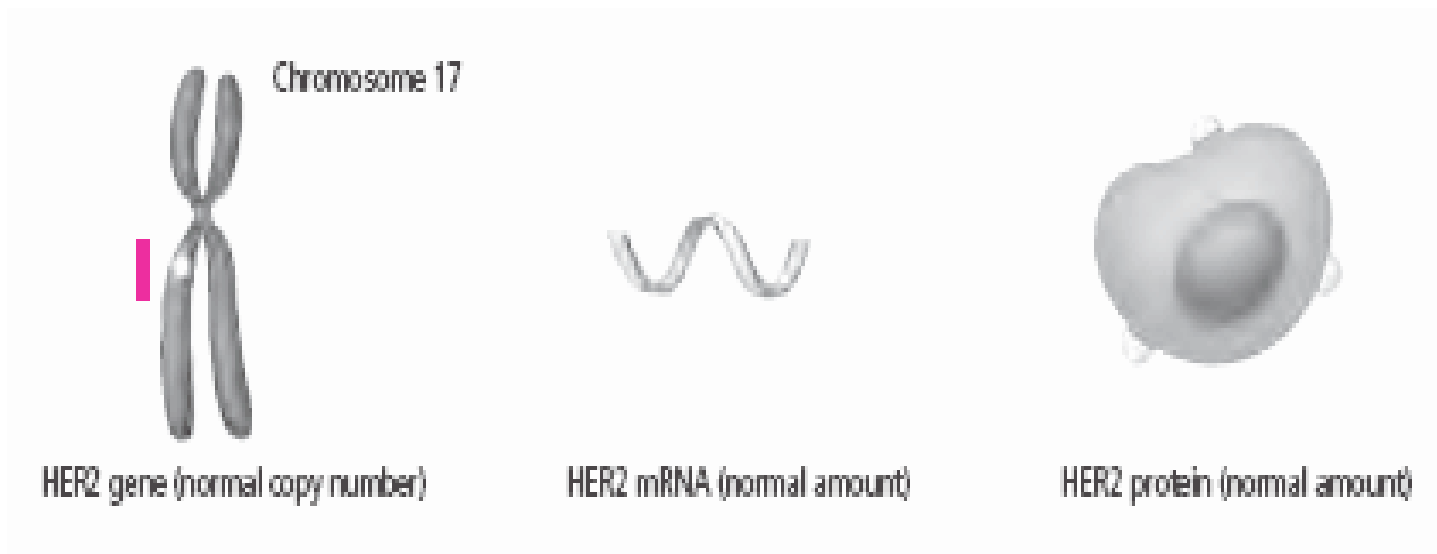
HER4

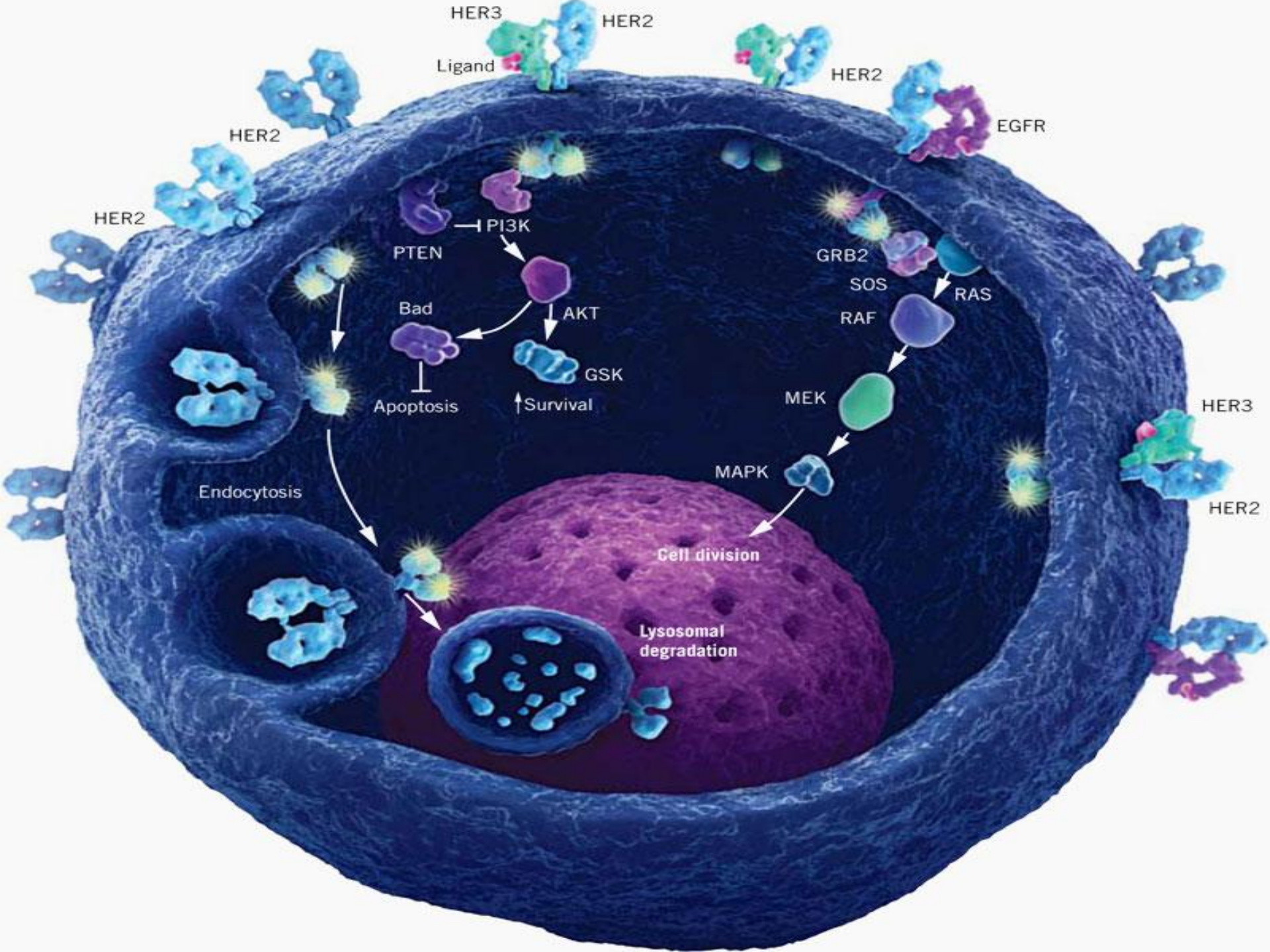


Intracellular ligand-binding domain

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- HER2 is a 185-kDa transmembrane **oncoprotein** (p185) encoded by **HER2/neu** gene located on **chromosome 17 (q21.1)**.





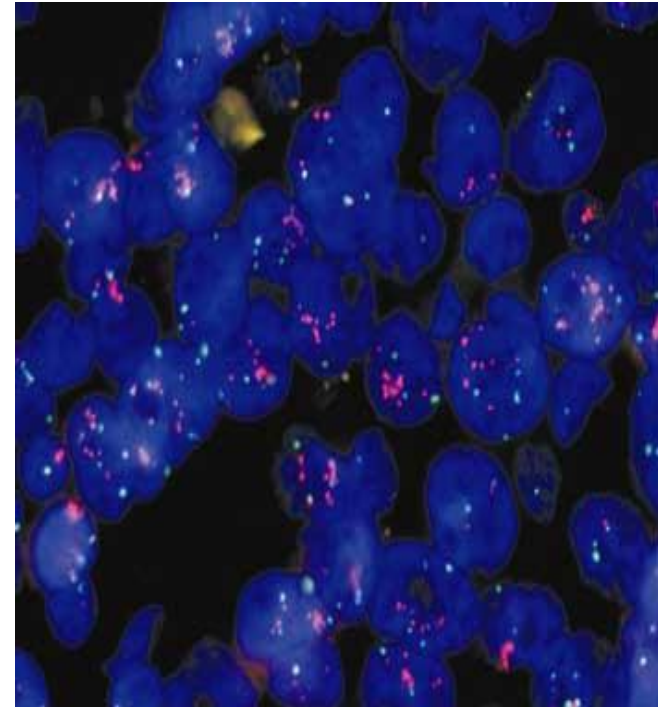


HER-2 & Effective Treatment

- HER2 receptor is **overexpressed in 20–30 %** of BC.



- **Trastuzumab (Herceptin) IgG1**, a monoclonal antibody, has been approved by FDA to be anti-HER2 therapy.



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Trastuzumab Resistance

- Almost **50 %**
do not
respond to
Trastuzumab-
based therapy.



- **Primary.**

- **Eventually.**

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Value

- Trastuzumab costs about **US\$70,000** for a full course of treatment.
- Trastuzumab brought = **\$327 million** in revenue for Genentech in the **fourth quarter** of 2007.

- Ref.

- Fleck L (2006). "The costs of caring: Who pays? Who profits? Who panders?". *Hastings Cent Rep* **36** (3): 13–7.
- ["Will Herceptin Media Blitz Help Or Harm Patients?"](#). Scoop. 2006-02-14. Retrieved 2006-12-01.

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AIM



Determination of the most probable mechanism/s of resistance

Accurate diagnosis of the implicated resistance mechanism in each subset of patients

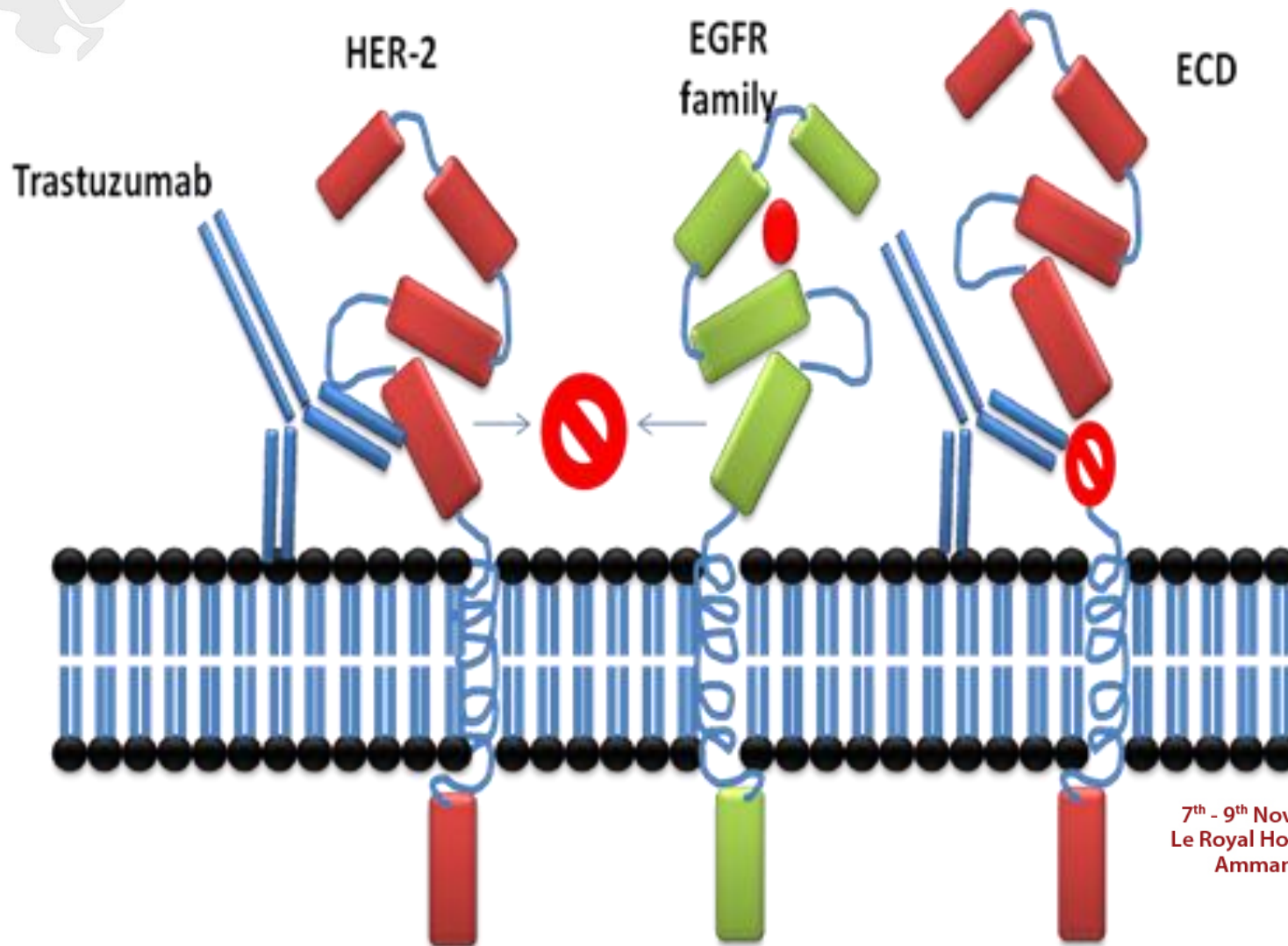
Design of more efficient treatment protocols.



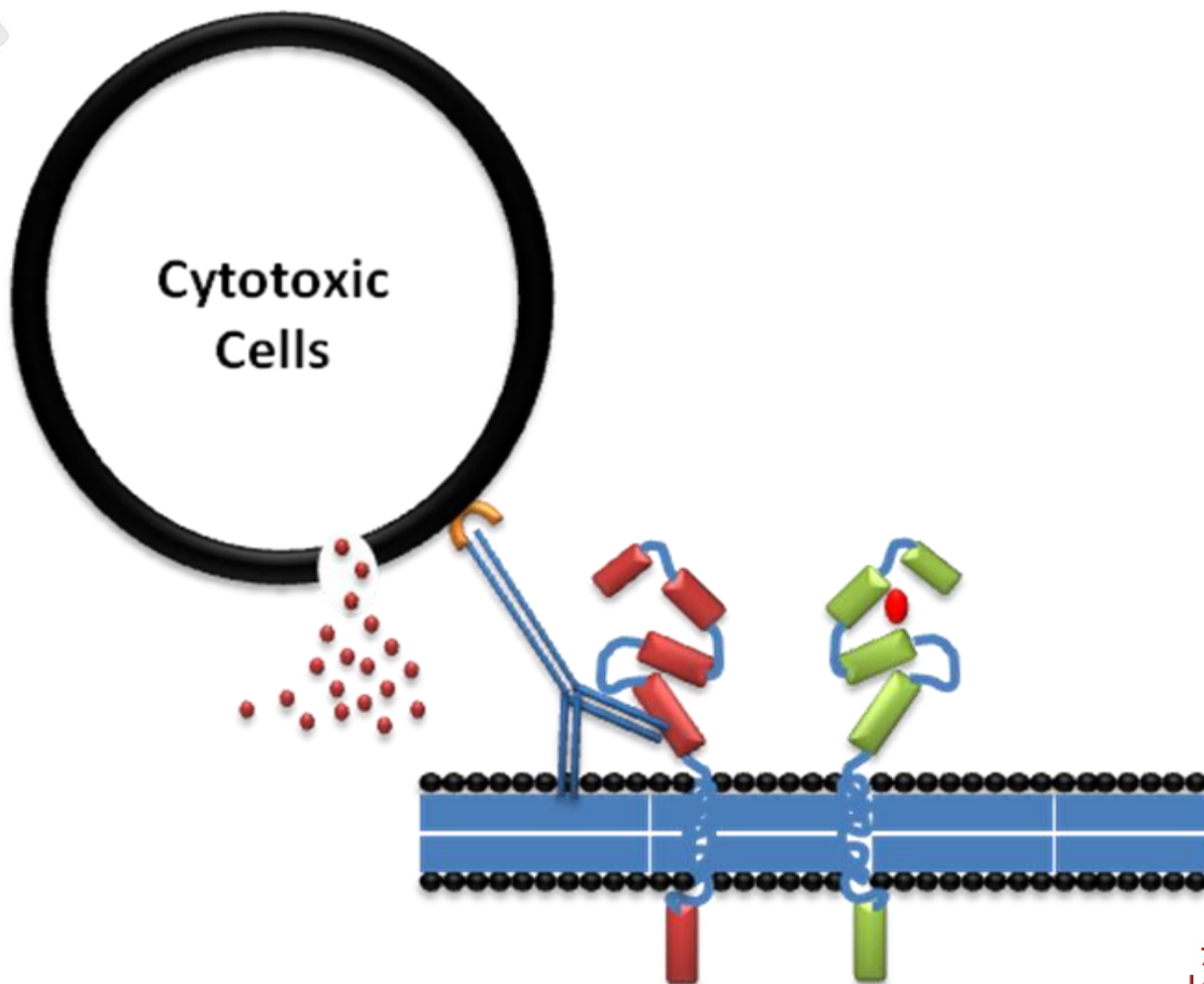
Trastuzumab

Mechanism of Action

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Trastuzumab Resistance

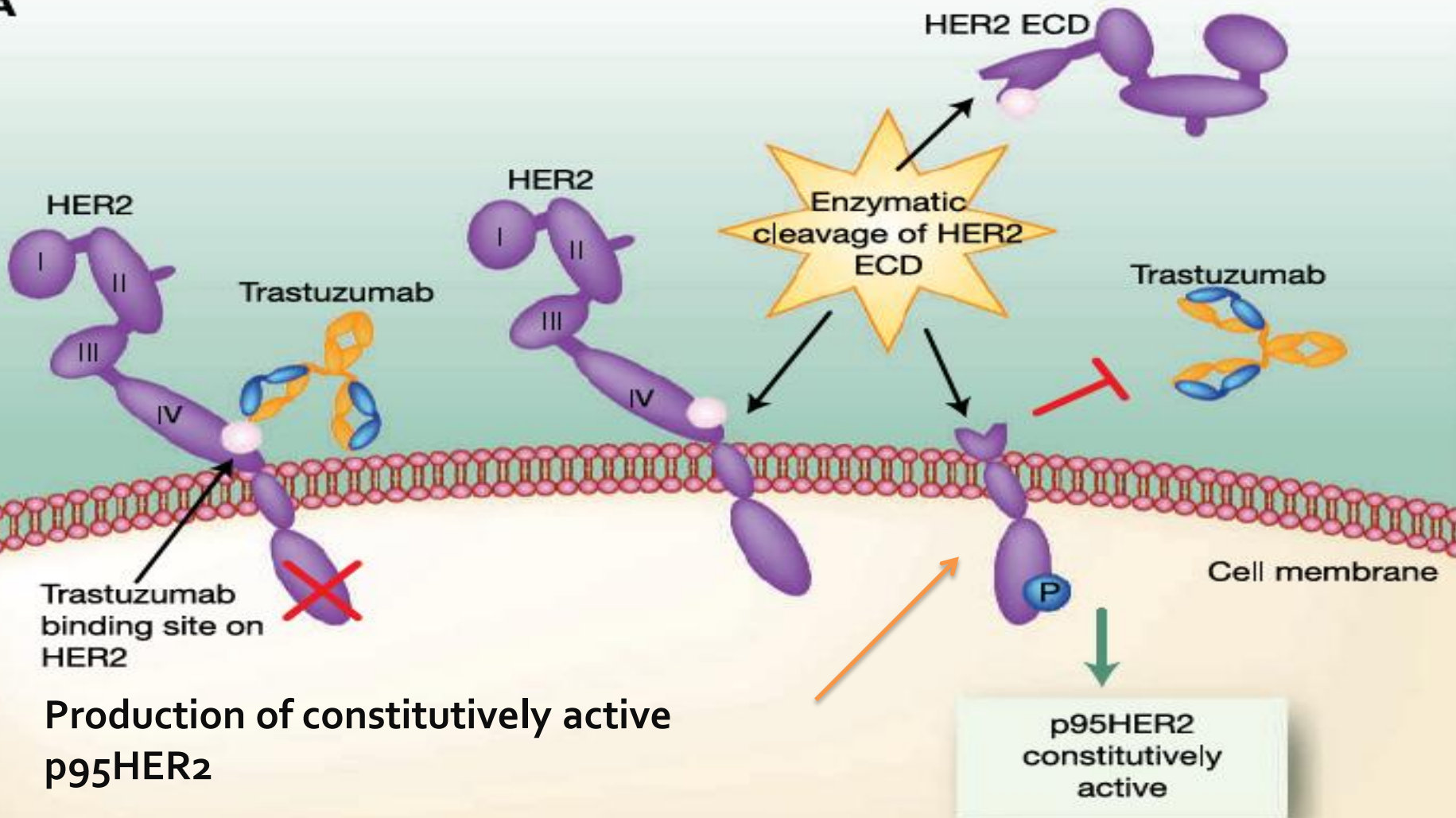


Proposed Mechanisms of Trastuzumab Resistance

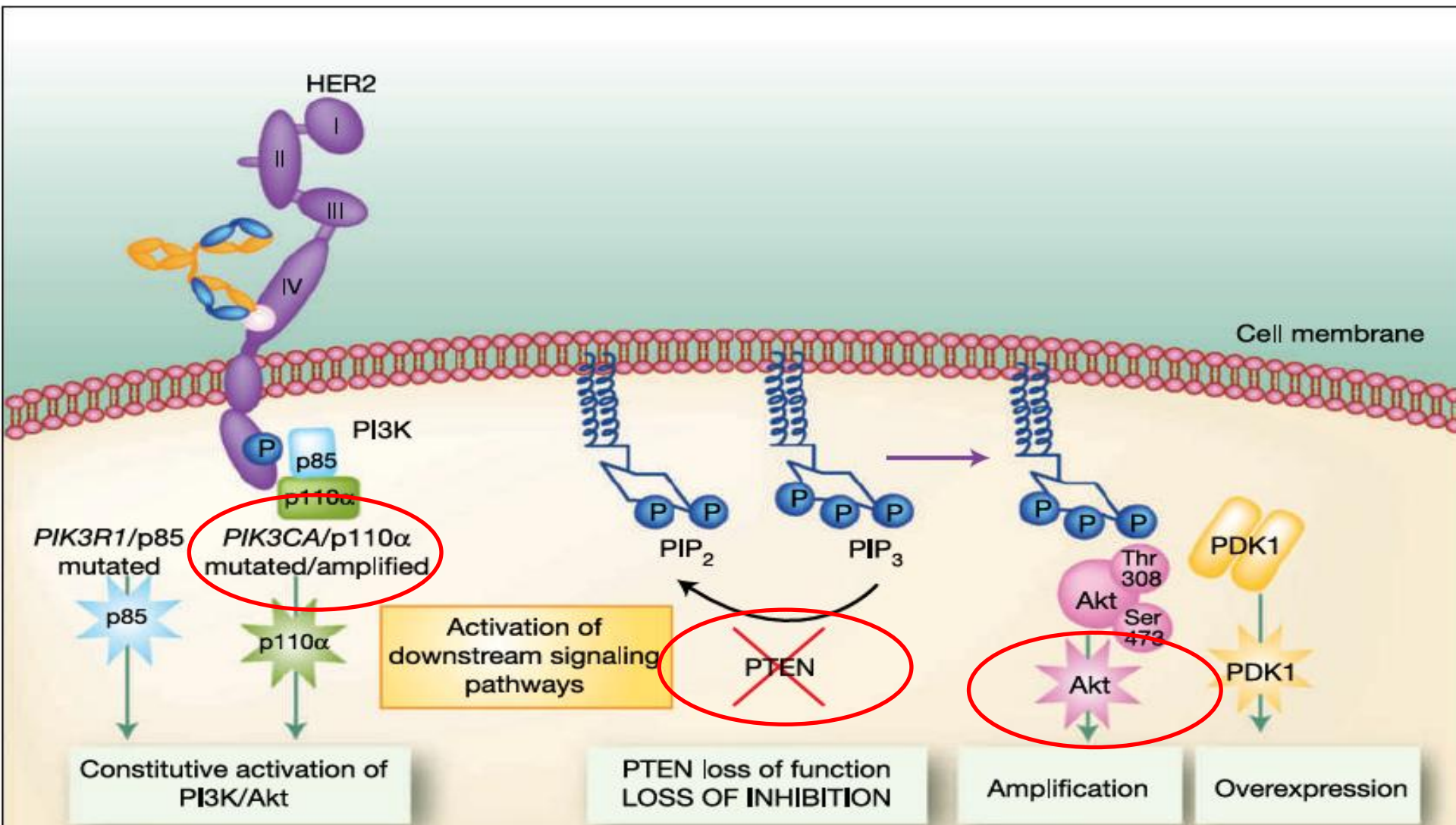
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1. Obstacles for Trastuzumab Binding to HER-2:

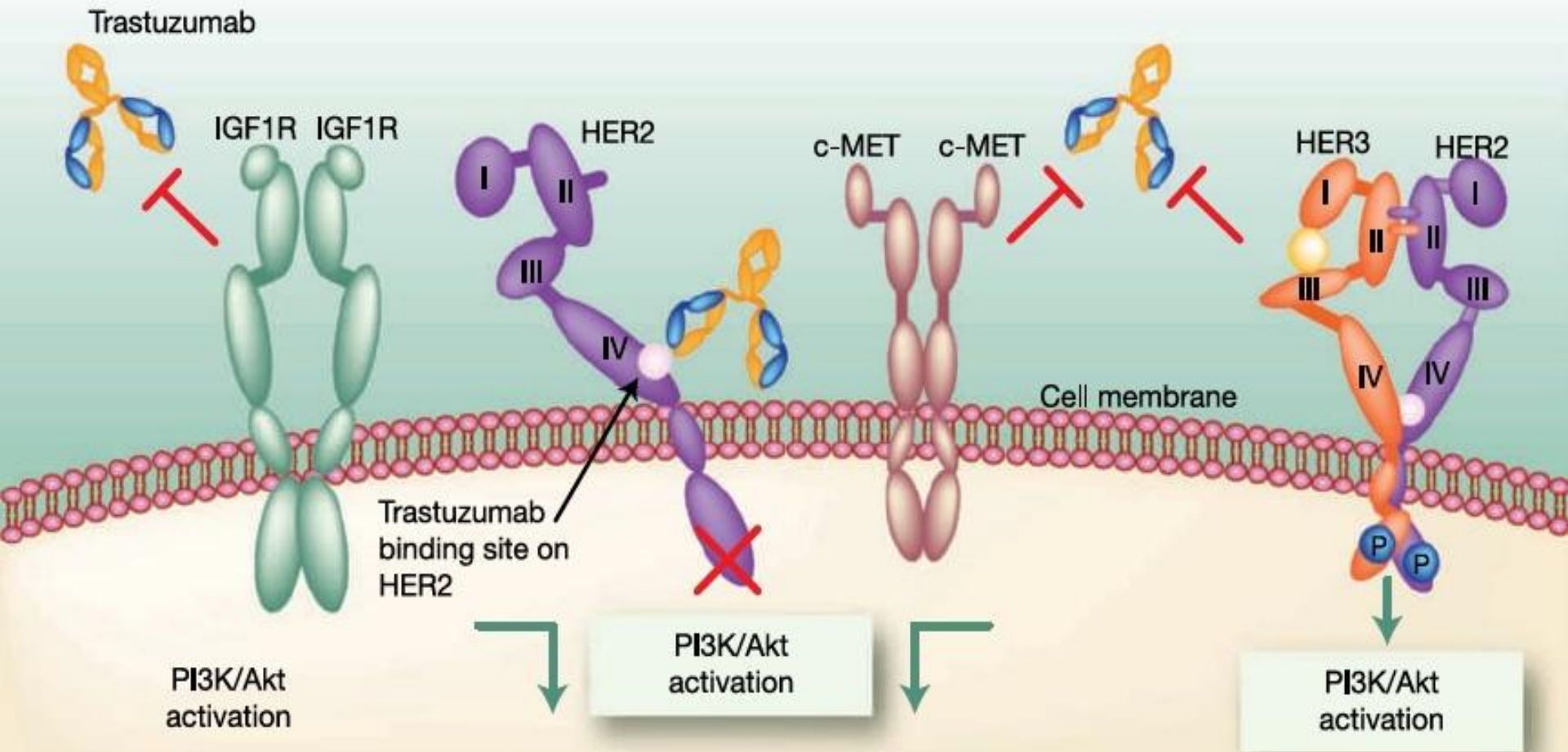
A



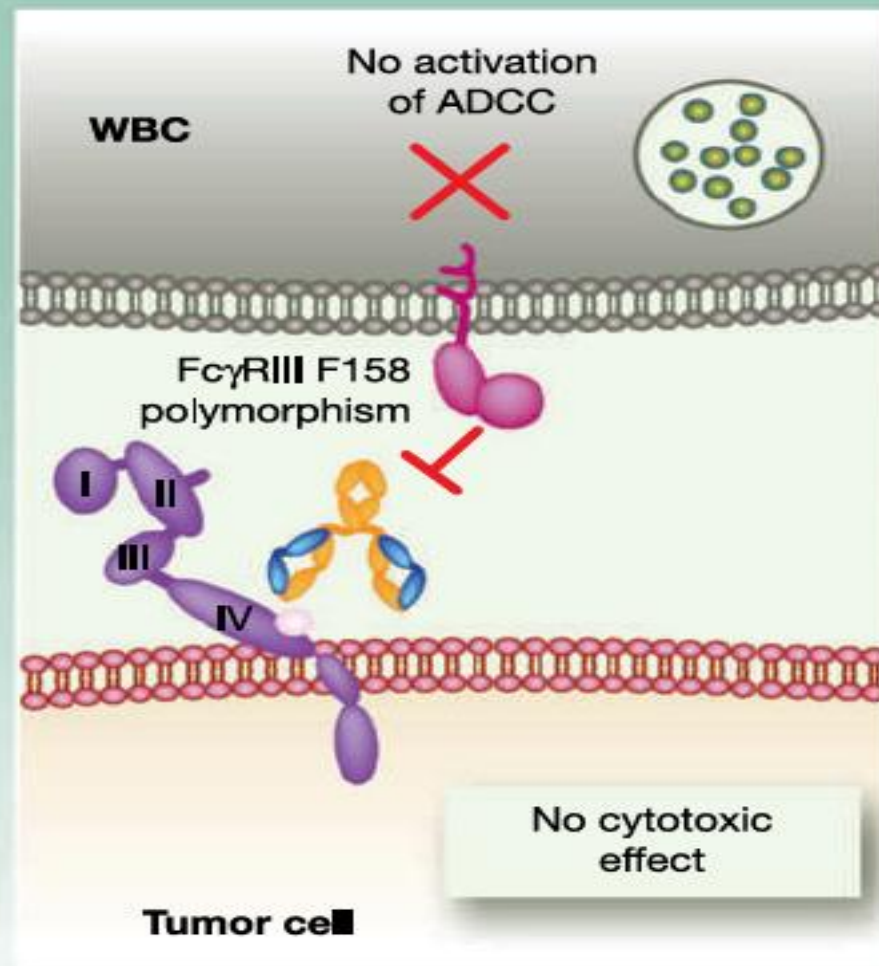
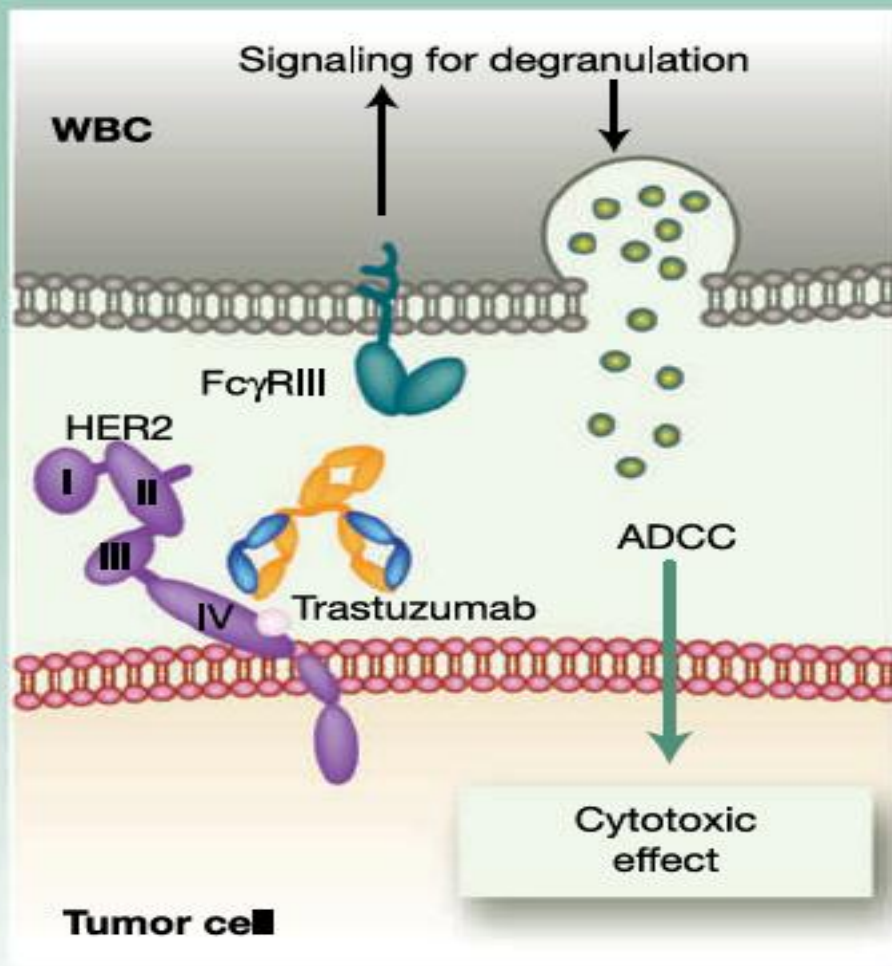
2. Upregulation of HER2 signaling pathways:



3. Signaling through alternative pathways



4. Failure to trigger ADCC





Study Population

- **77 females** with metastatic breast cancer overexpressing **HER-2**.
- Average Age of Diagnosis: **55** years.
 - **45 %** < 55 years
 - **55 %** > 55 years

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Gene	Exon	No. Of Mutation	% of Mutations	Total %	Total %	Total %
PIK3CA	9	7/77	9 %	21 %	24 %	38 %
	20	9/77	12 %			
AKT1	4	2/77	3 %	3 %		
PTEN	5, 7 and 8	14/77	18 %	18 %	18 %	
HER-2	17-24	0	0	0	0	

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Her2/neu Mutations

- Her2 Mutations are **not found** in

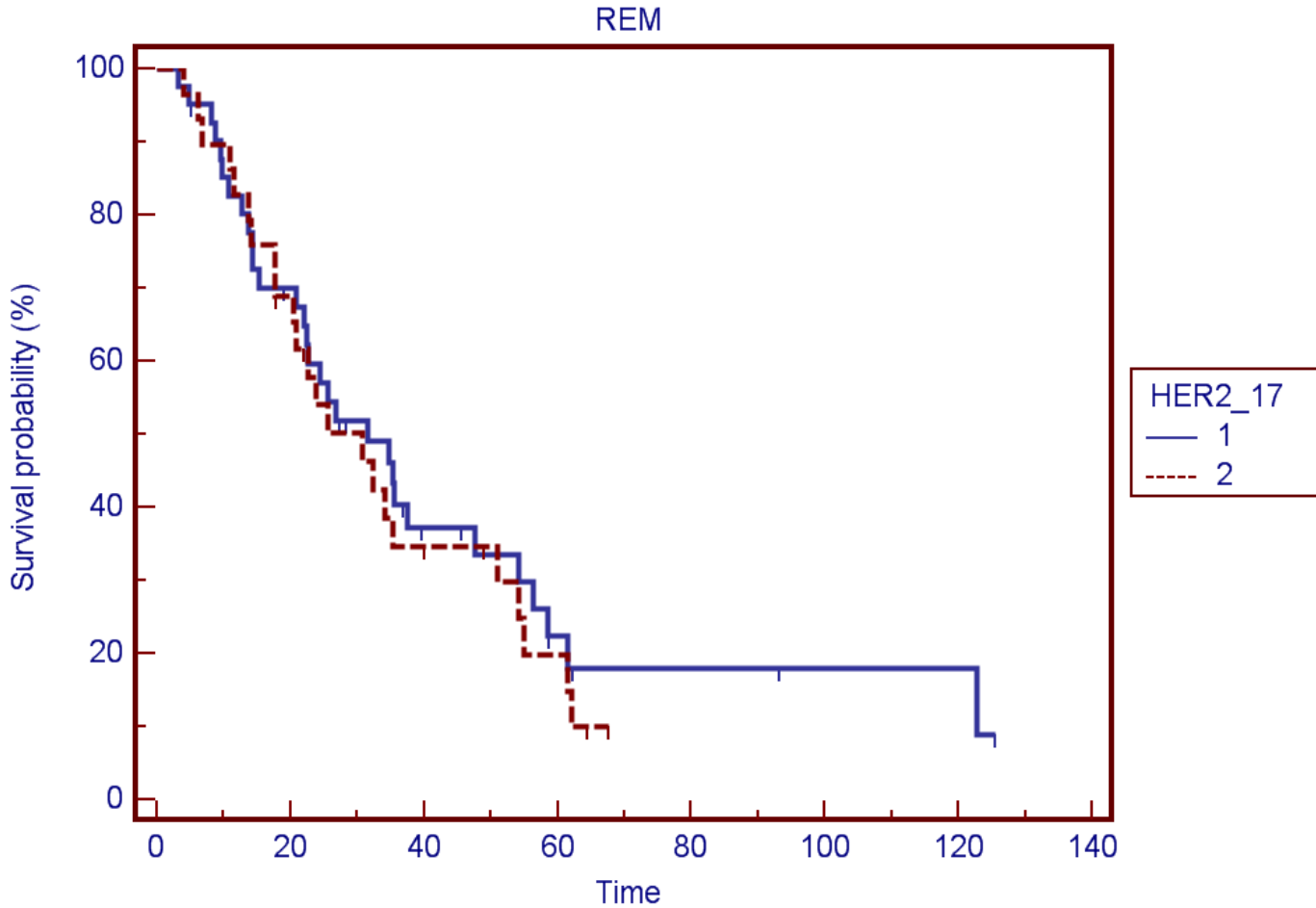
exons 17 - 24

- Exon 17 SNP has been noticed.

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Exon 17 SNP



Number at risk

Group: 1

41 27 11 5 3 2 2 1

Group: 2

29 19 9 4 0 0 0 0

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PIK3CA mutations

- Exon 9 mutations = 7 / 77 → 9 %
- Exon 20 mutations = 9 / 77 → 13 %
- Mutation in either Exons = 15/77
21 %

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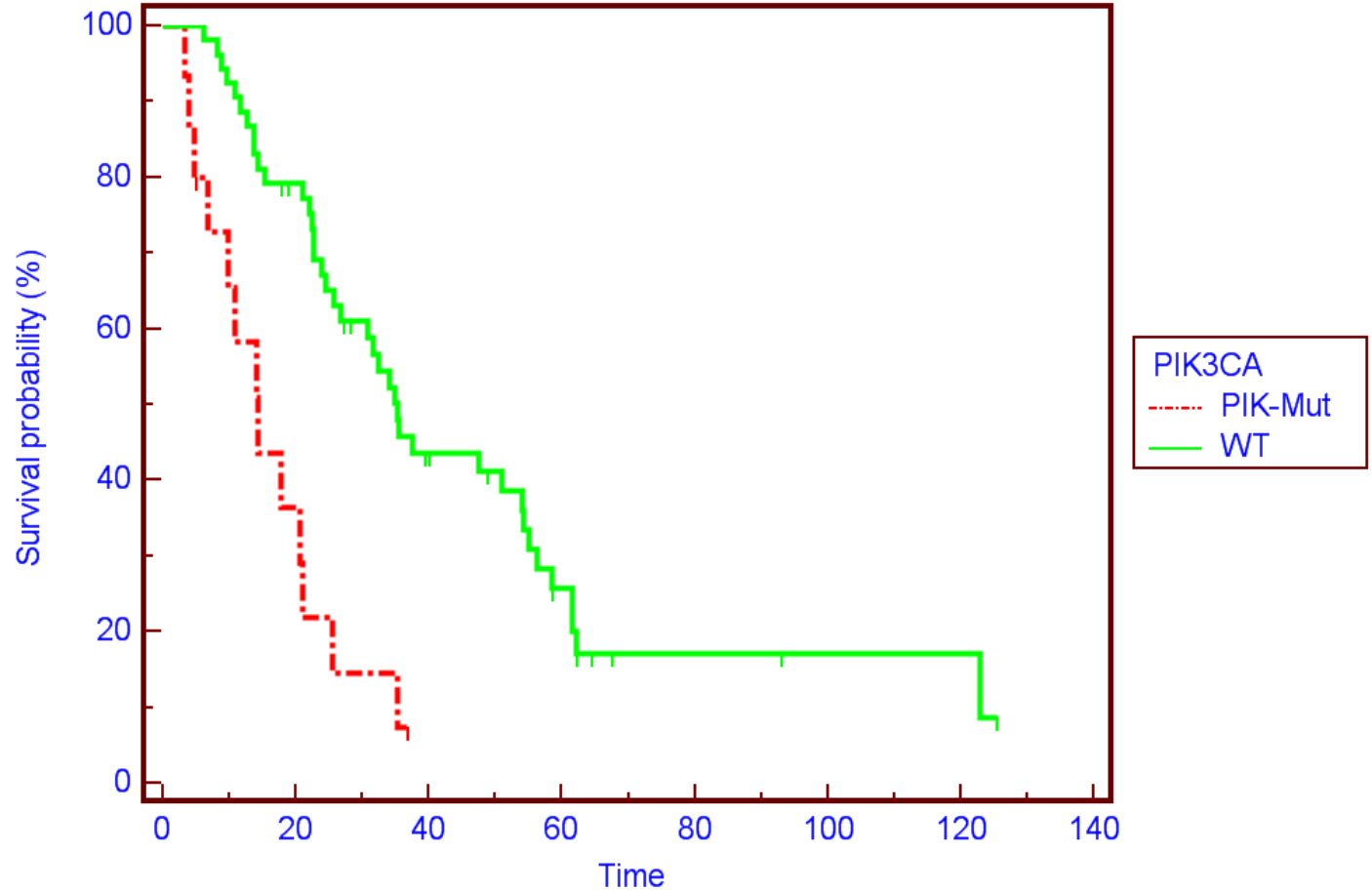
- **Akt mutations**
 - **Exon 4 = 2 / 77**

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OverAll Survival (OS)



REM



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Number at risk

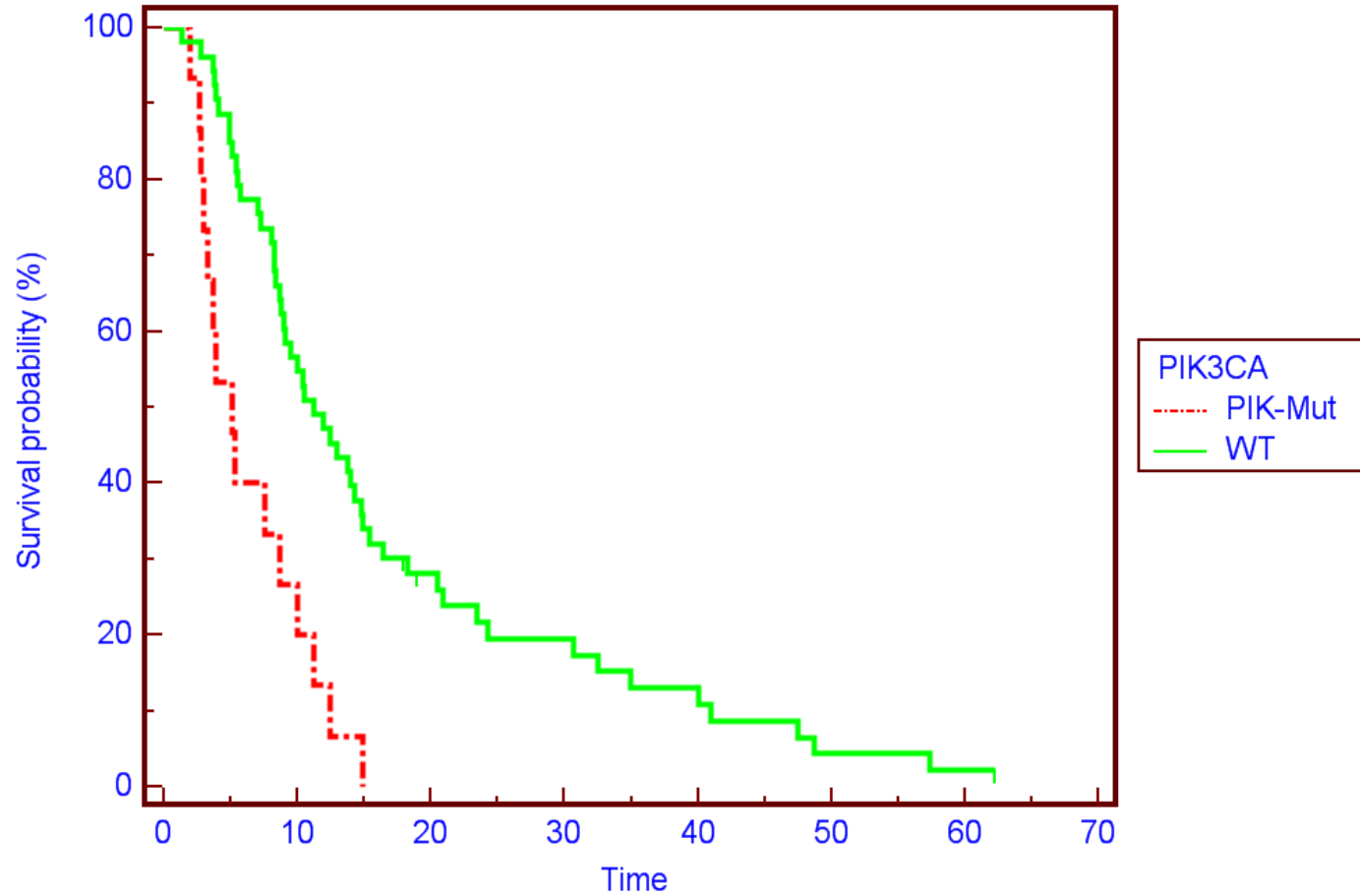
Time	0	5	10	15	20	25	30	35	40
Group: PIK-Mut	15	5	0	0	0	0	0	0	0
Group: WT	53	40	19	9	3	2	2	2	1



PFS-I



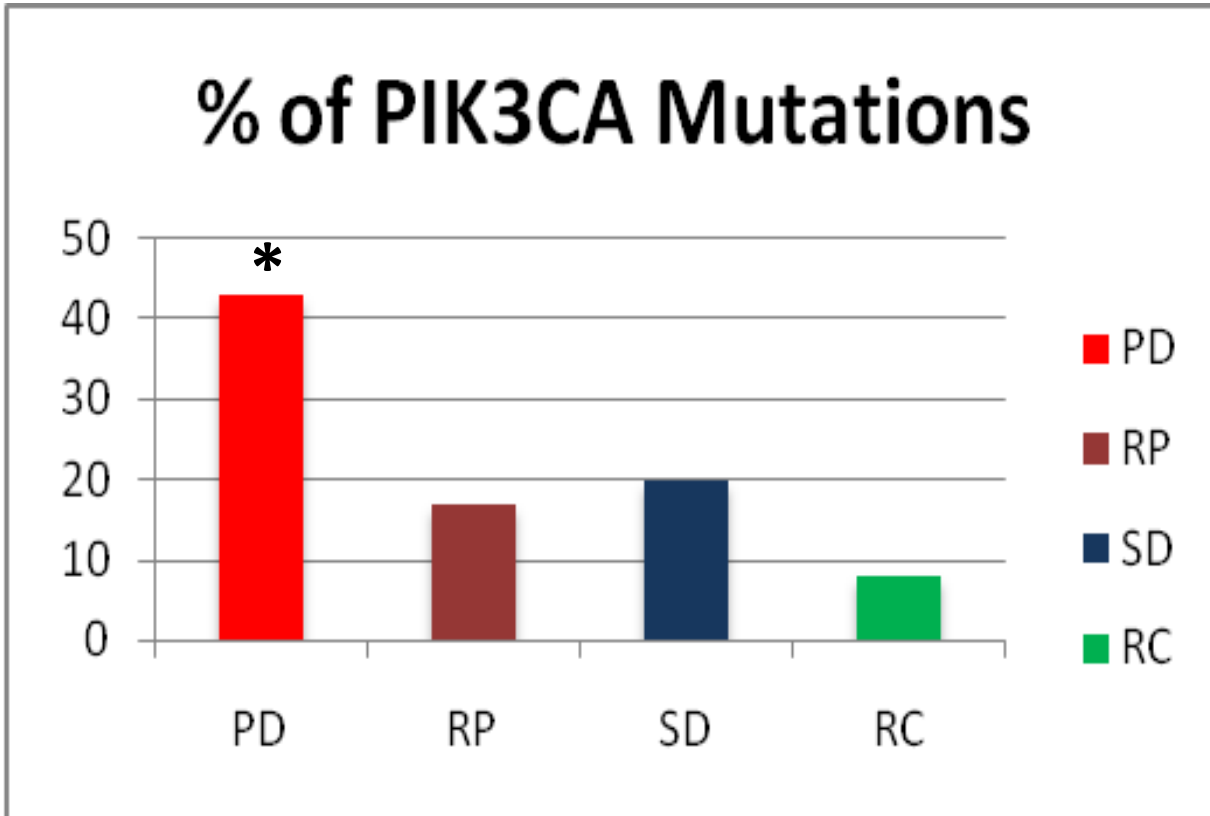
REM1



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Number at risk

Time	0	5	10	15	20	25	30	35	40	45	50	55	60	65
Group: PIK-Mut	15	3	0	0	0	0	0	0	0	0	0	0	0	0
Group: WT	53	29	13	9	5	2	1	1						



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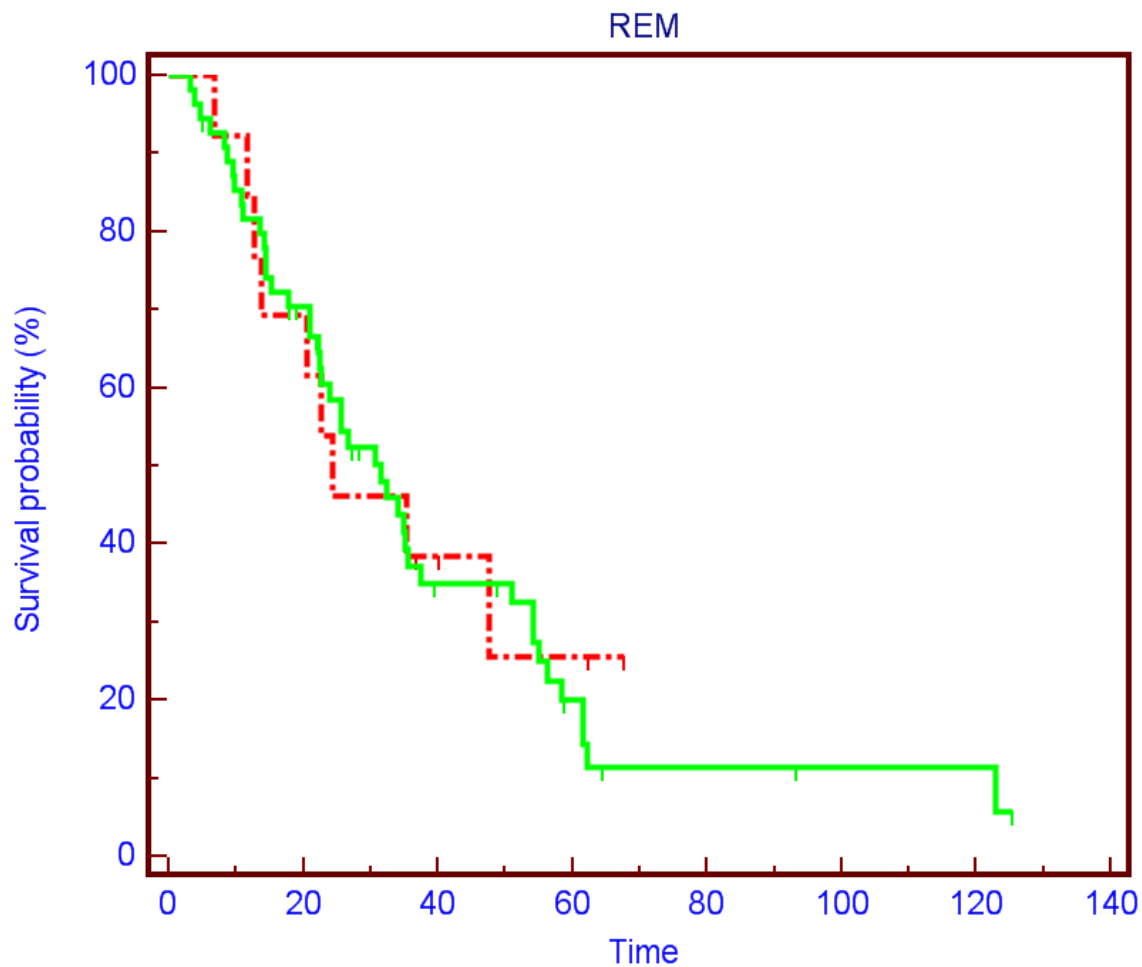
PTEN Mutations Analysis

- Exon 5 → $7/77 = 10.2 \%$
- Exon 7 → $9/77 = 13.2 \%$
- Exon 8 → $2/77 = 2.9 \%$
- 5 patients have more than one mutation
- Total Mutated Patients $14/77 = 18 \%$

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OS



Number at risk
Group: PTEN-Mut

13 9 4 2 0 0 0 0

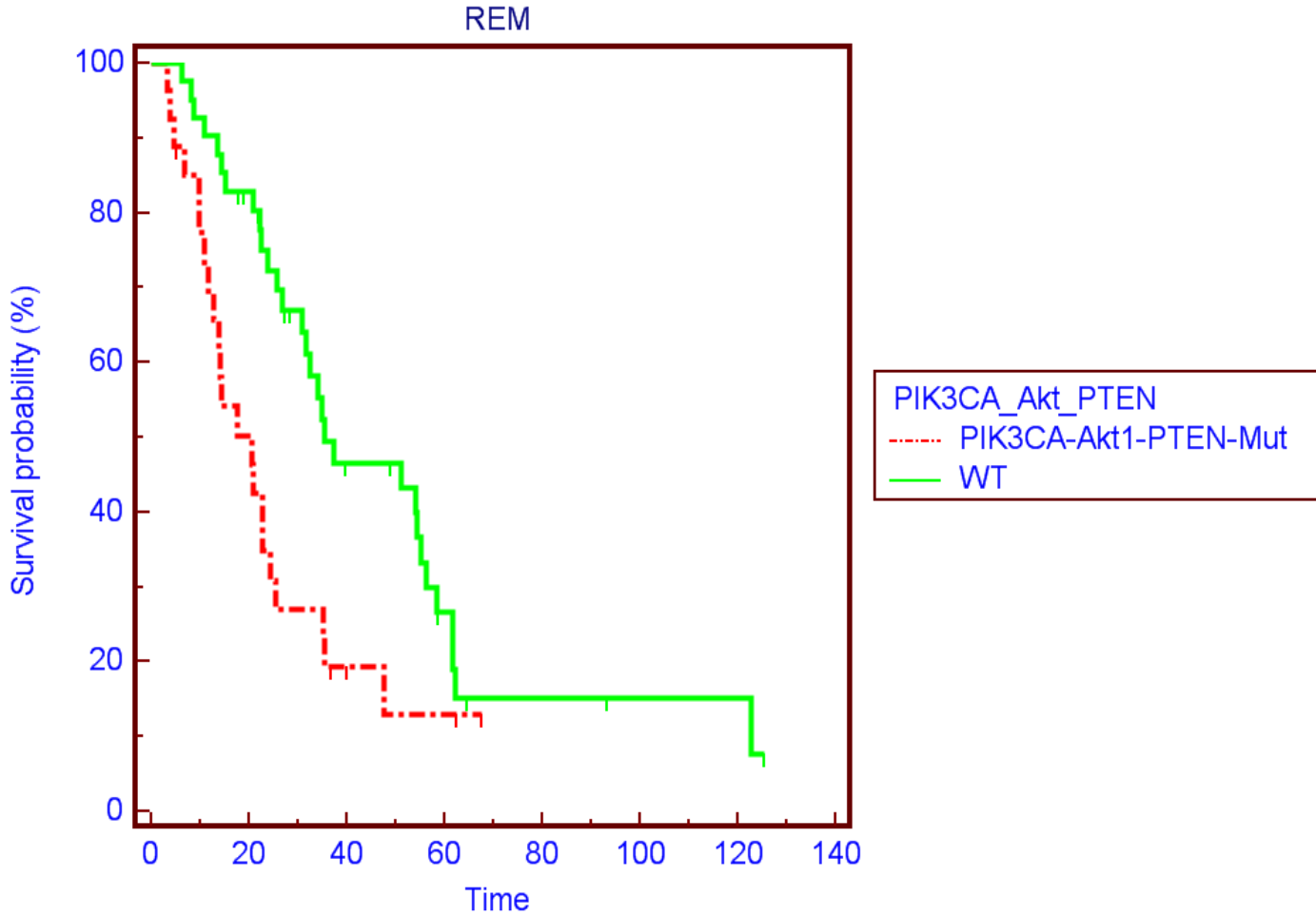
Group: WT

55 36 15 7 3 2 2 1

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OS



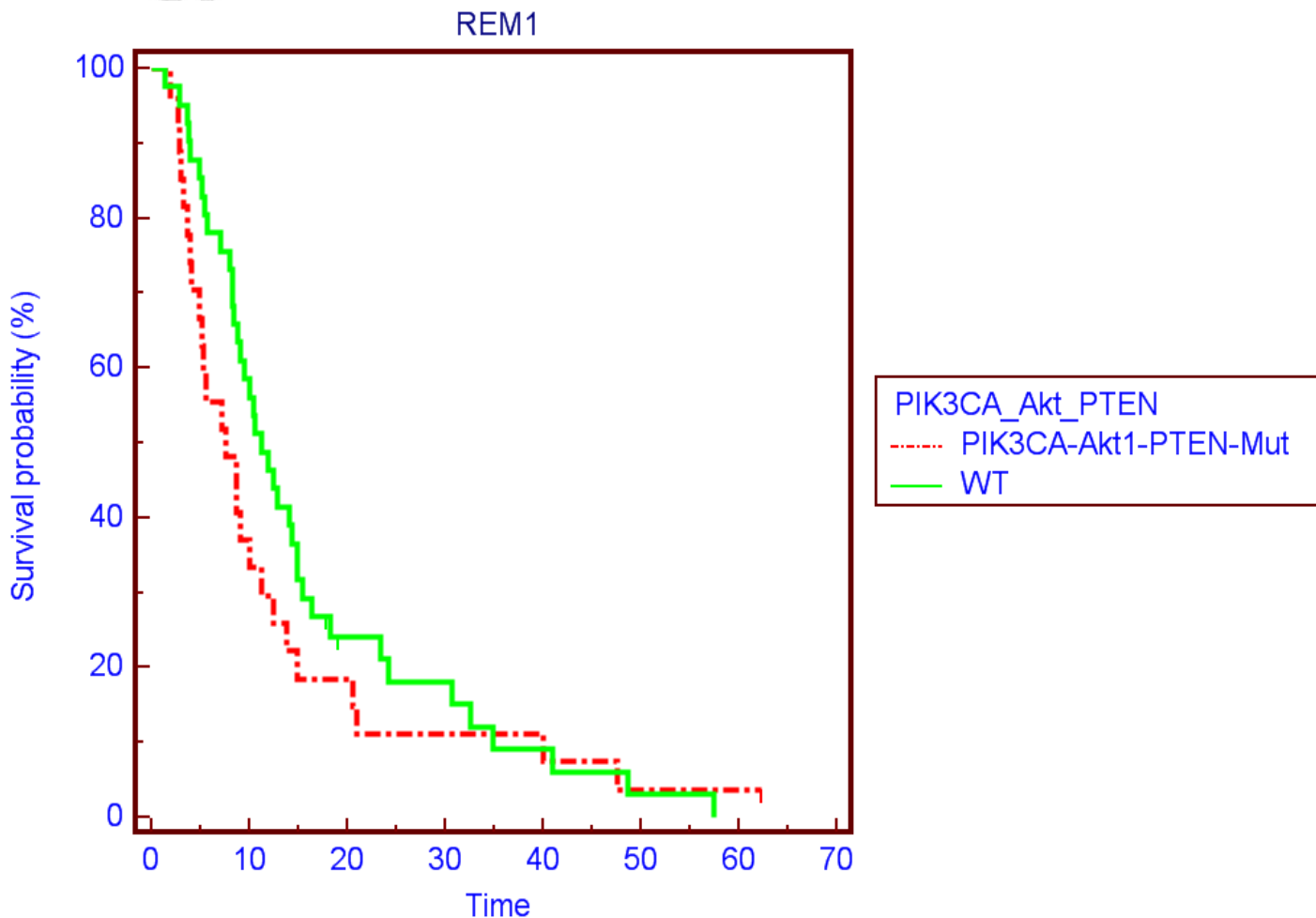
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Number at risk

Group	0	10	20	30	40	50	60	70	80	90	100	110	120	130
Group: PIK3CA-Akt1-PTEN-Mut	27	13	4	2	0	0	0	0	0	0	0	0	0	0
Group: WT	41	32	15	7	3	2	2	2	1					



PFS-I



Number at risk

Group: PIK3CA-Akt1-PTEN-Mut

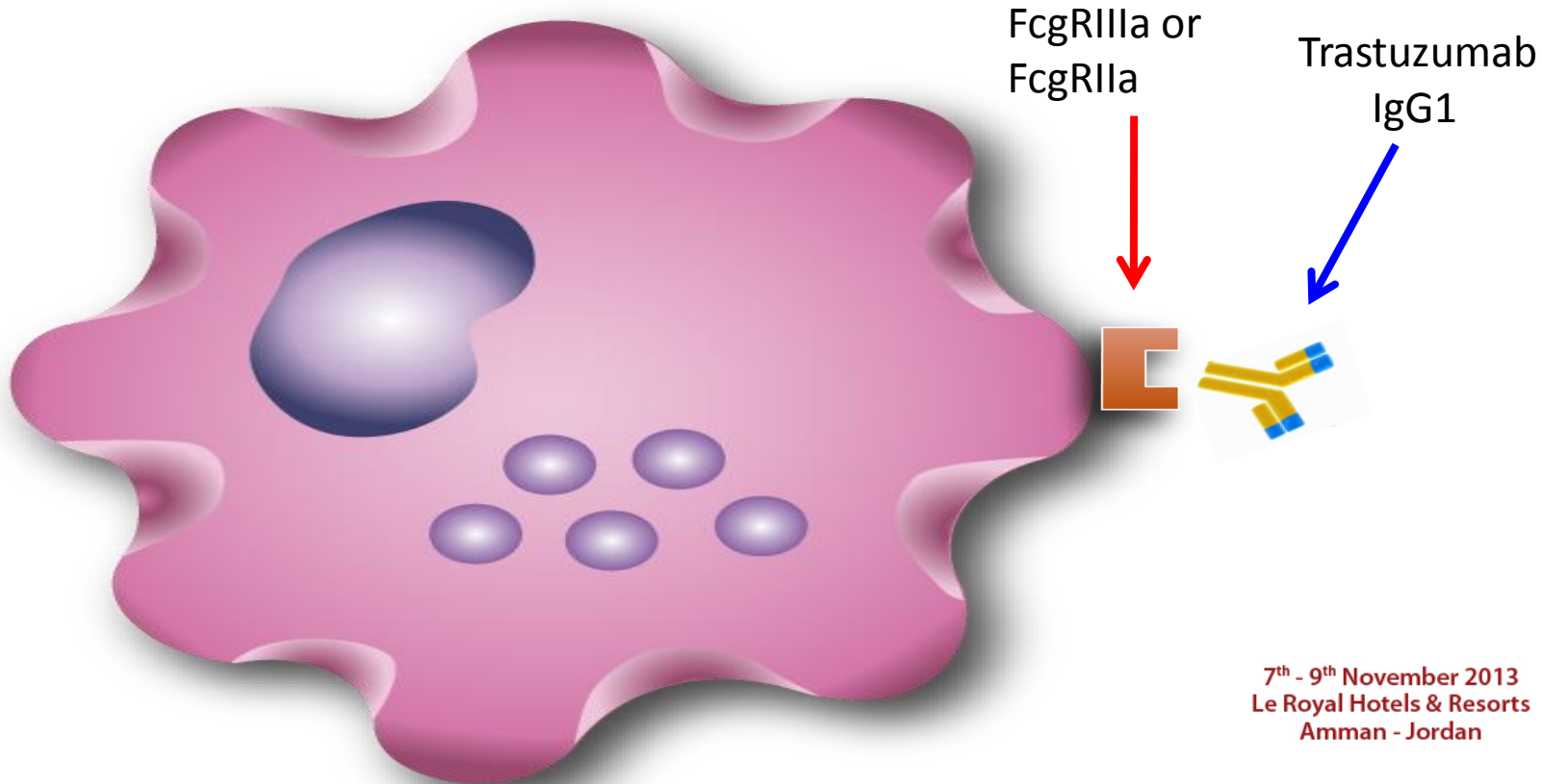
27 9 5 3 2 1 1 1

Group: WT

41 23 8 6 3 1 0 0

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FcgRIIIa F158V and FcgRIIa R131H SNPs



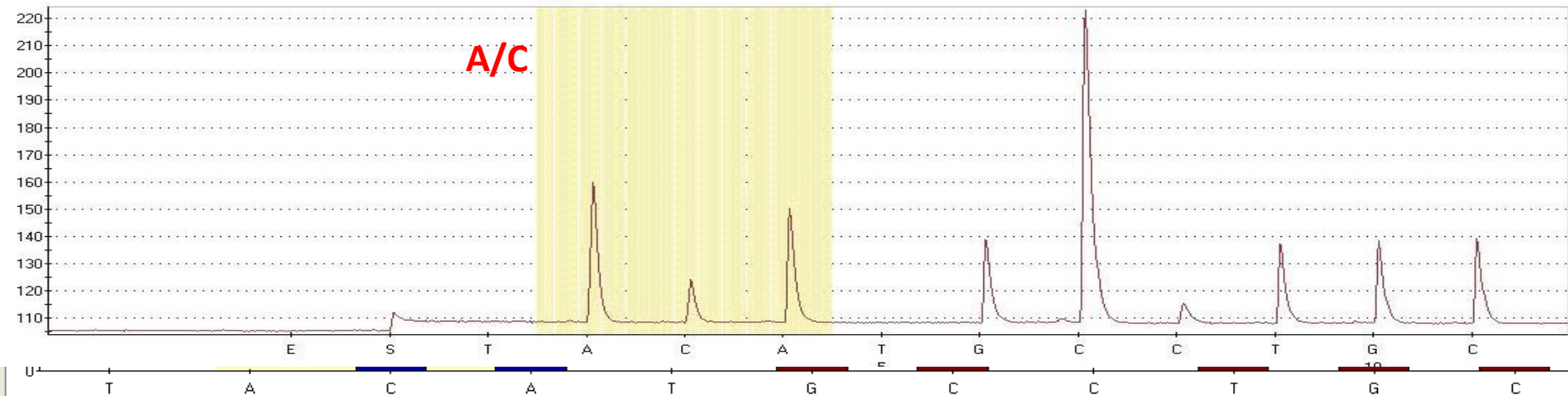
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FcgRIIIA-1: A1

A/C

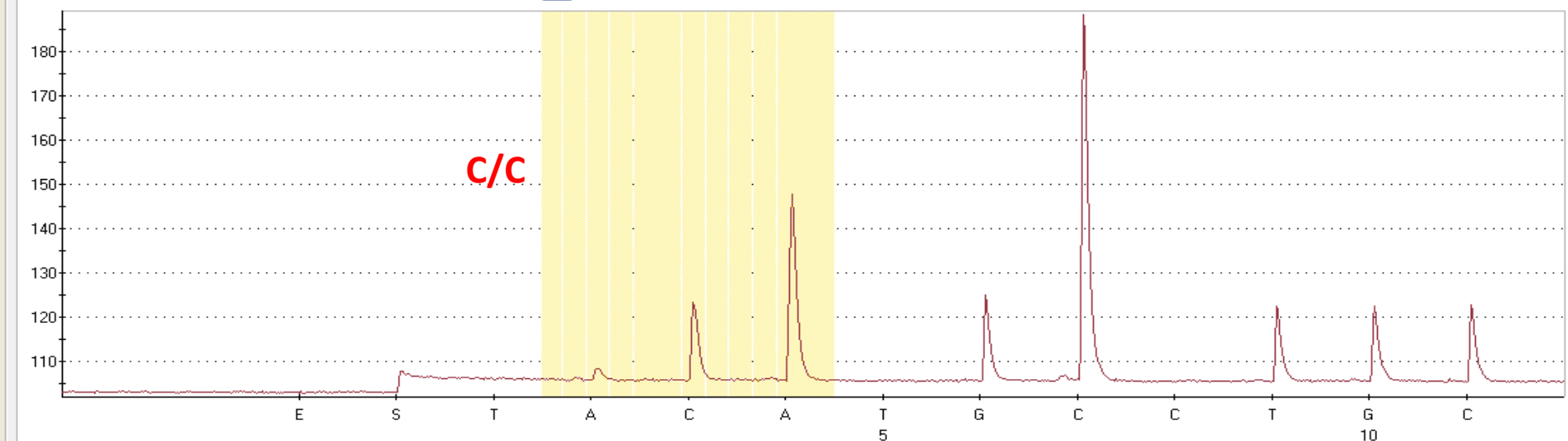
A/C



FcgRIIIA-1: B4

C/C

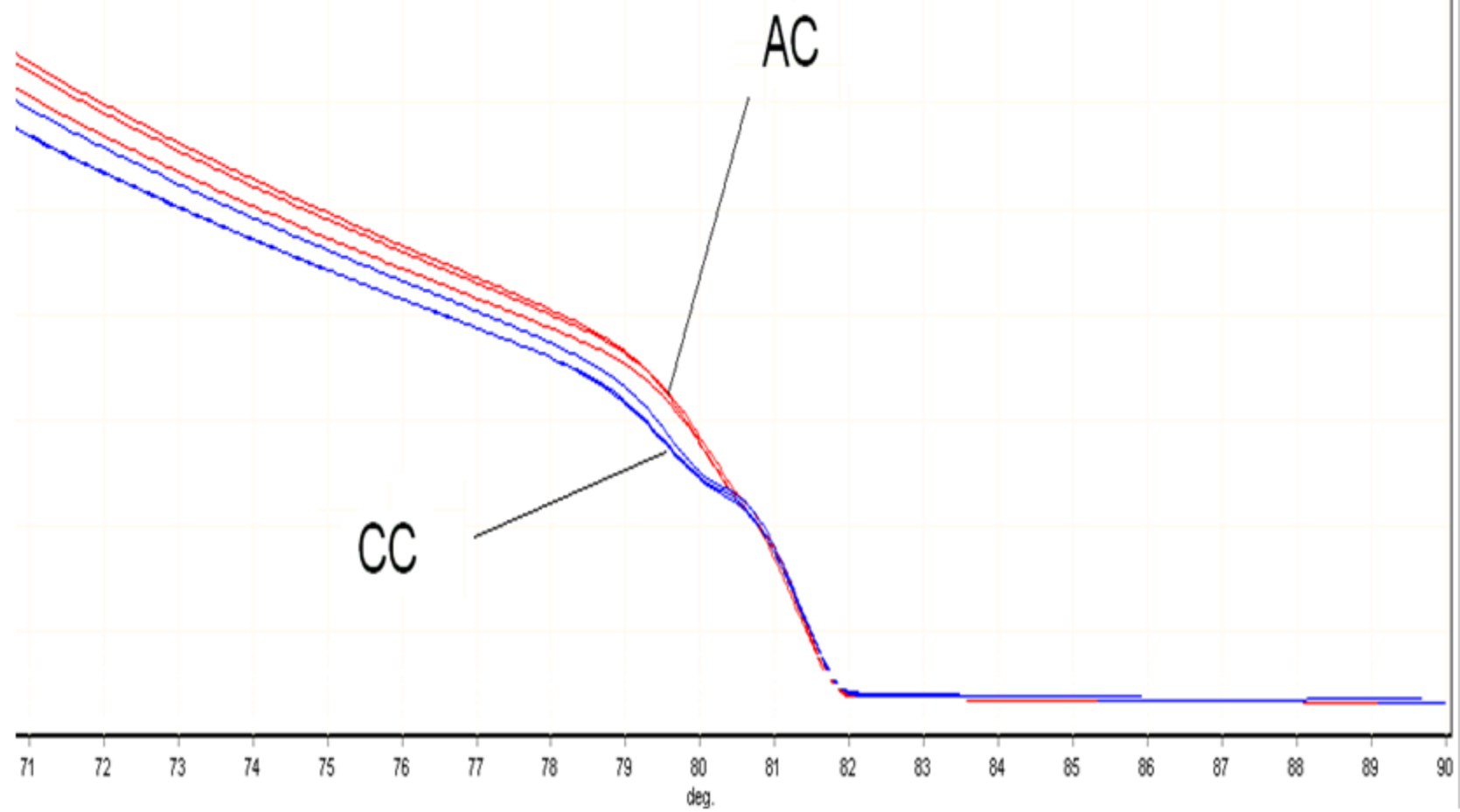
C/C

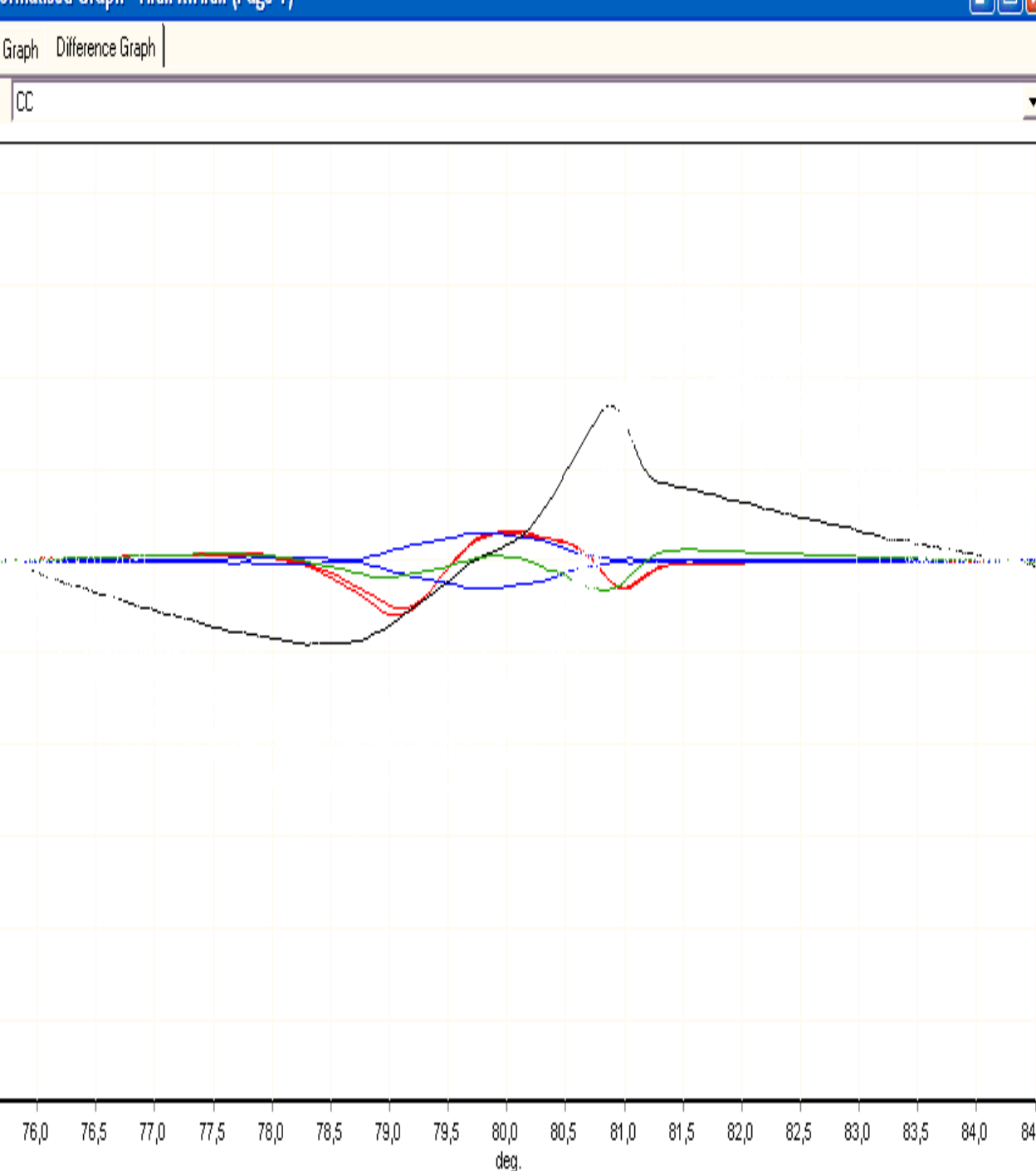


High Resolution Melting (HRM)

1	4
2	5
3	6
4	10
5	10
6	10
7	NC
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	

Bank On	Bank Off	
Named On	All On	All Off
Edit Samples...		





No.	C	Name	Genotype	Confidence
1	AC	AC	AC	99,72
2	AC	AC	AC	99,72
3	CC	CC	CC	83,90
4	CC	CC	CC	83,90
5	AA	AA	AA	100,00
9	36	CC	CC	84,46
13				
14				
15				
16				
17				
18				
19				
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25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				

1	AC
2	AC
3	CC
4	CC
5	AA
6	AA
7	14
8	33
9	36
10	40
11	NC
12	NC
13	
14	
15	
16	

Bank On Bank Off

Named On All On All Off

Edit Samples...

Normalisation Regions

Leading Range

Start: 75,5

End: 76

Trailing Range

Start: 84

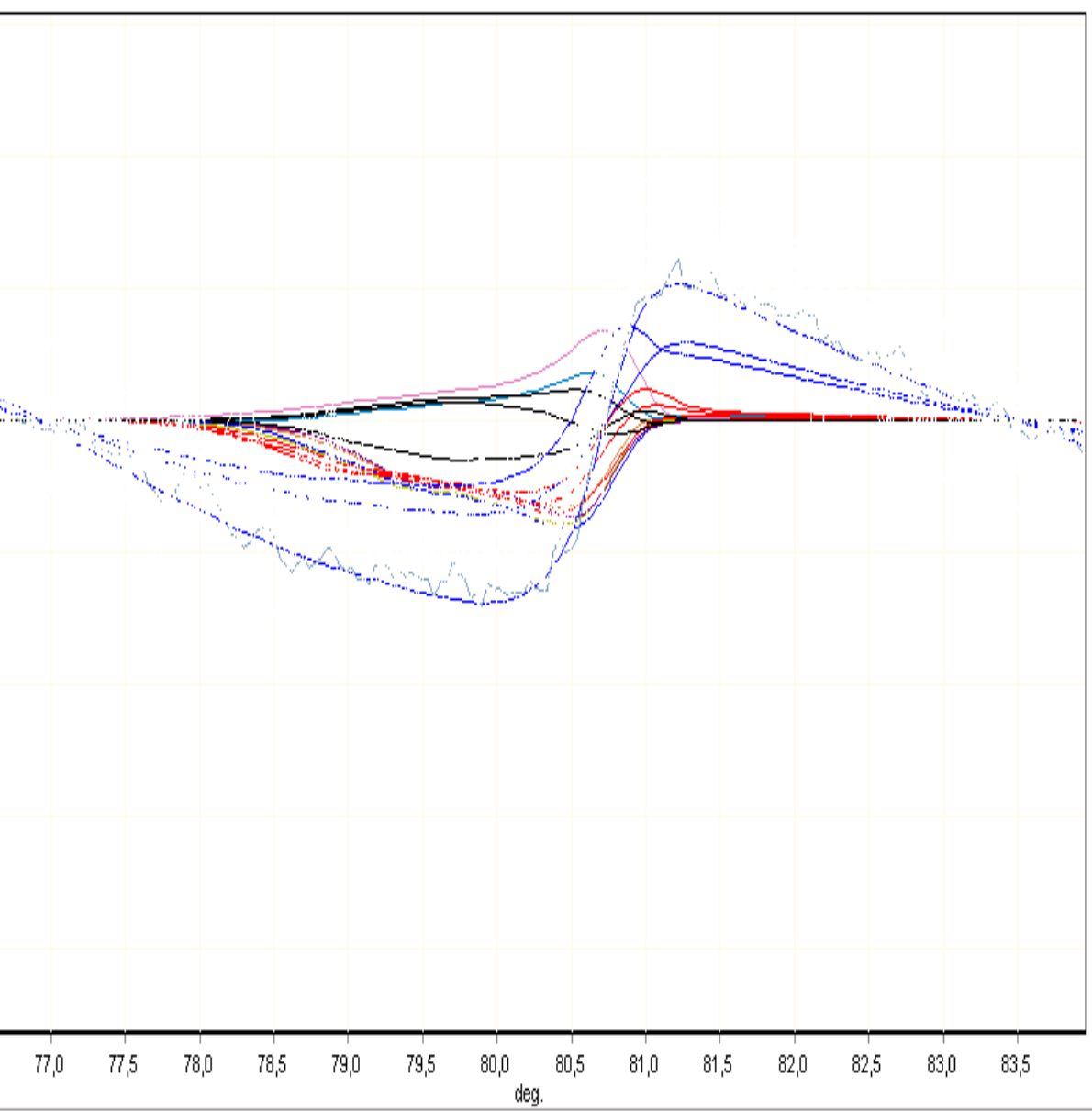
End: 84,5

Confidence Percentage

Threshold 1 %

Graph Difference Graph

CC



No.	C	Name	Genotype	Confidence %
1	2-4	AC	73,19	
2	2-5	AC	60,11	
3	2-6	AC	47,45	
4	2-7	AC	54,63	
5	2-8	Variation		
6	45	CC	46,08	
7	73	AC	99,43	
8	73	AC	80,44	
9	73	AC	79,46	
10	40	Variation		
11	40	CC	81,50	
12	40	CC	58,32	
13	56	AA	49,28	
14	56	Variation		
15	56	Variation		
16	NC	Variation		
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				

1	2-4
2	2-5
3	2-6
4	2-7
5	2-8
6	45
7	73
8	73
9	73
10	40
11	40
12	40
13	56
14	56
15	56
16	NC

Bank On Bank Off

Named On All On All Off

Edit Samples...

Normalisation Regions

Leading Range

Start: 76,46

End: 77,46

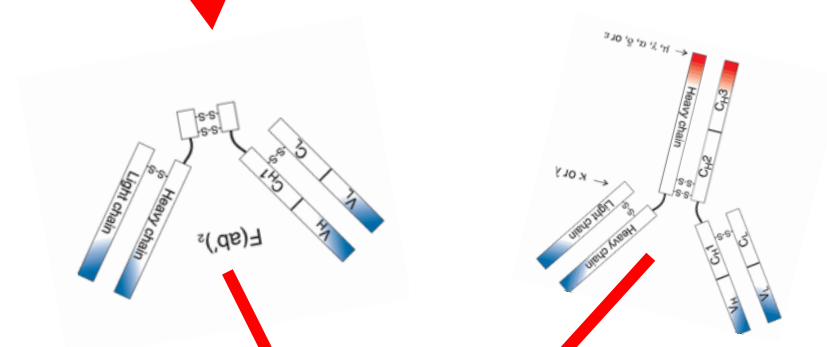
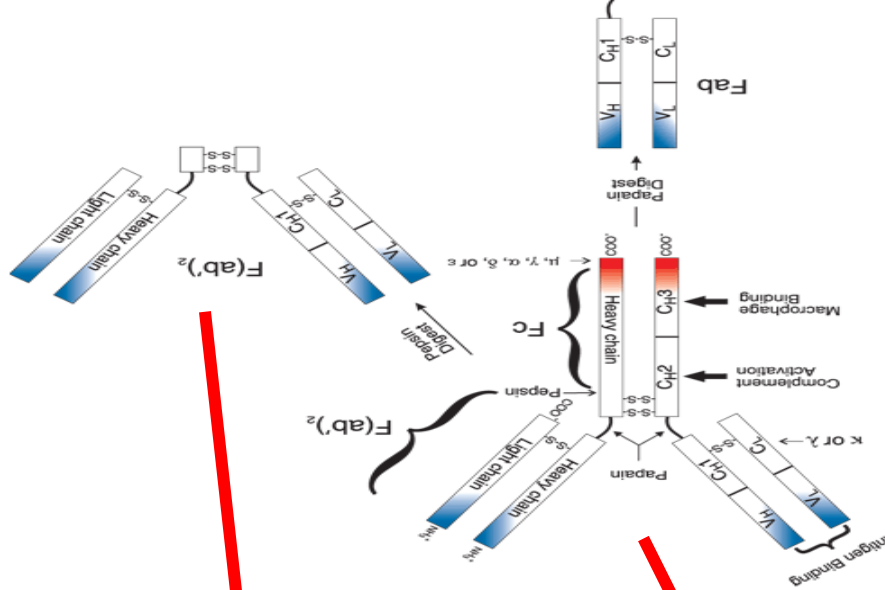
Trailing Range

Start: 82,99

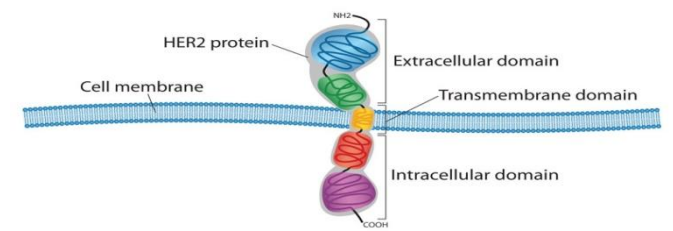
End: 83,99

Confidence Percentage

Threshold 40 %

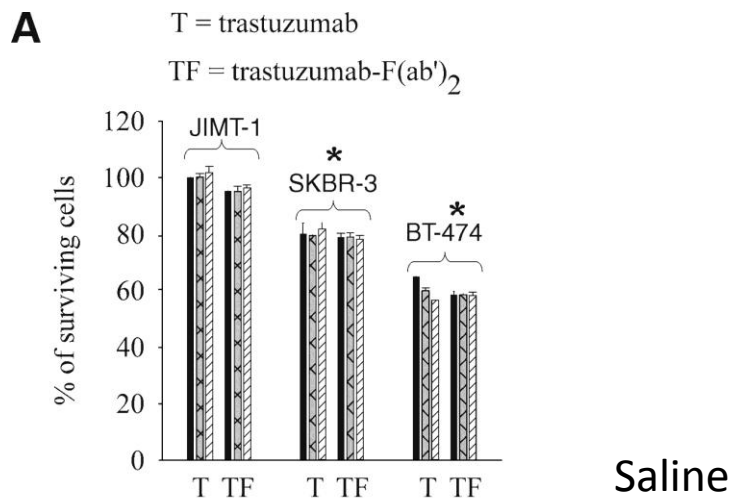


IgG and Fab attack equally

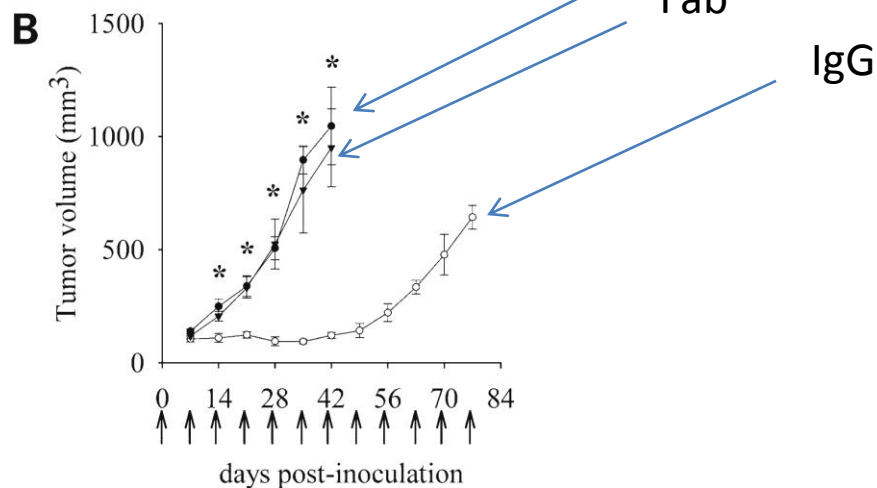


In vitro and in vivo effects of trastuzumab-F(ab')₂.

In vitro

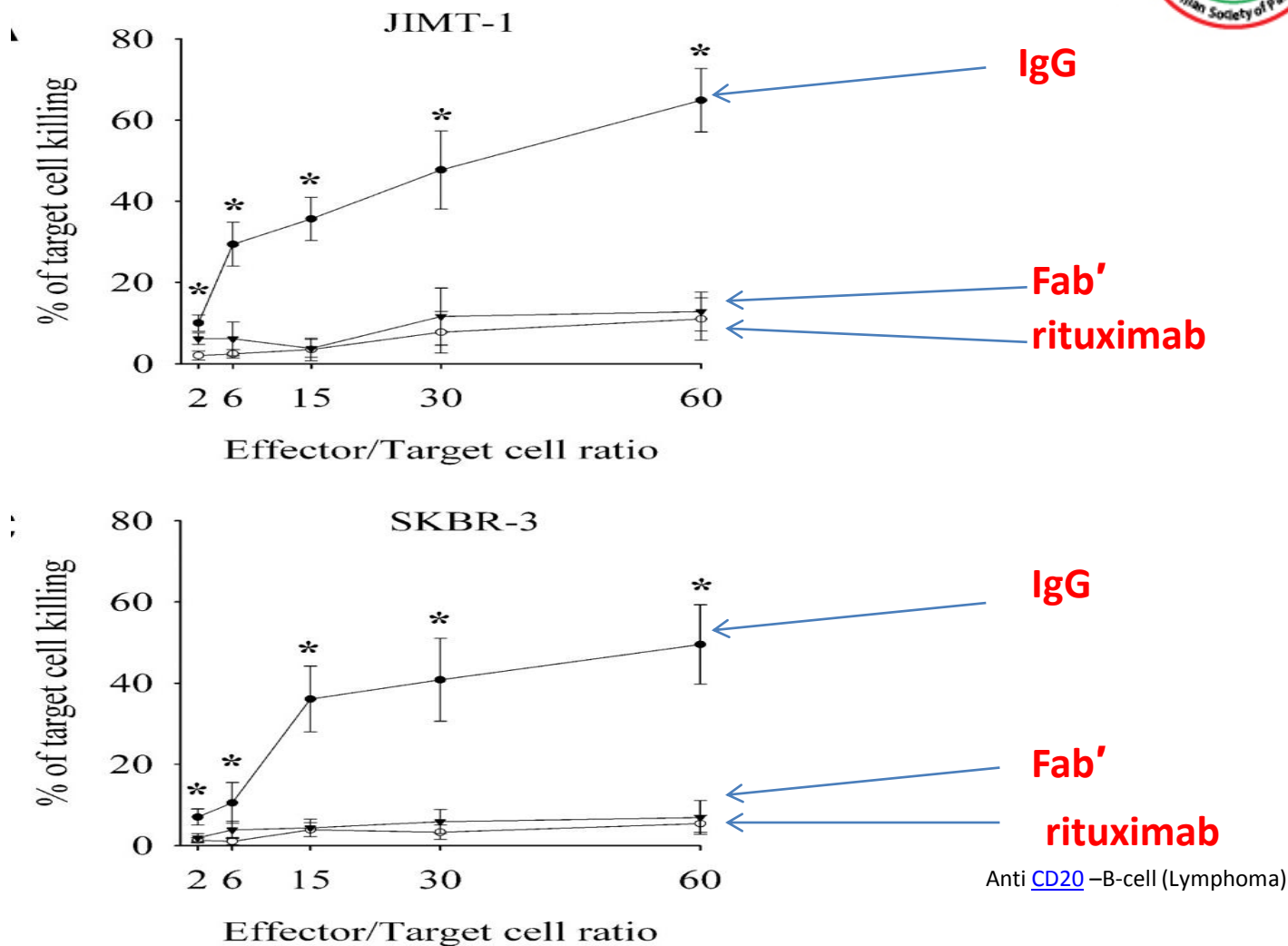


In vivo

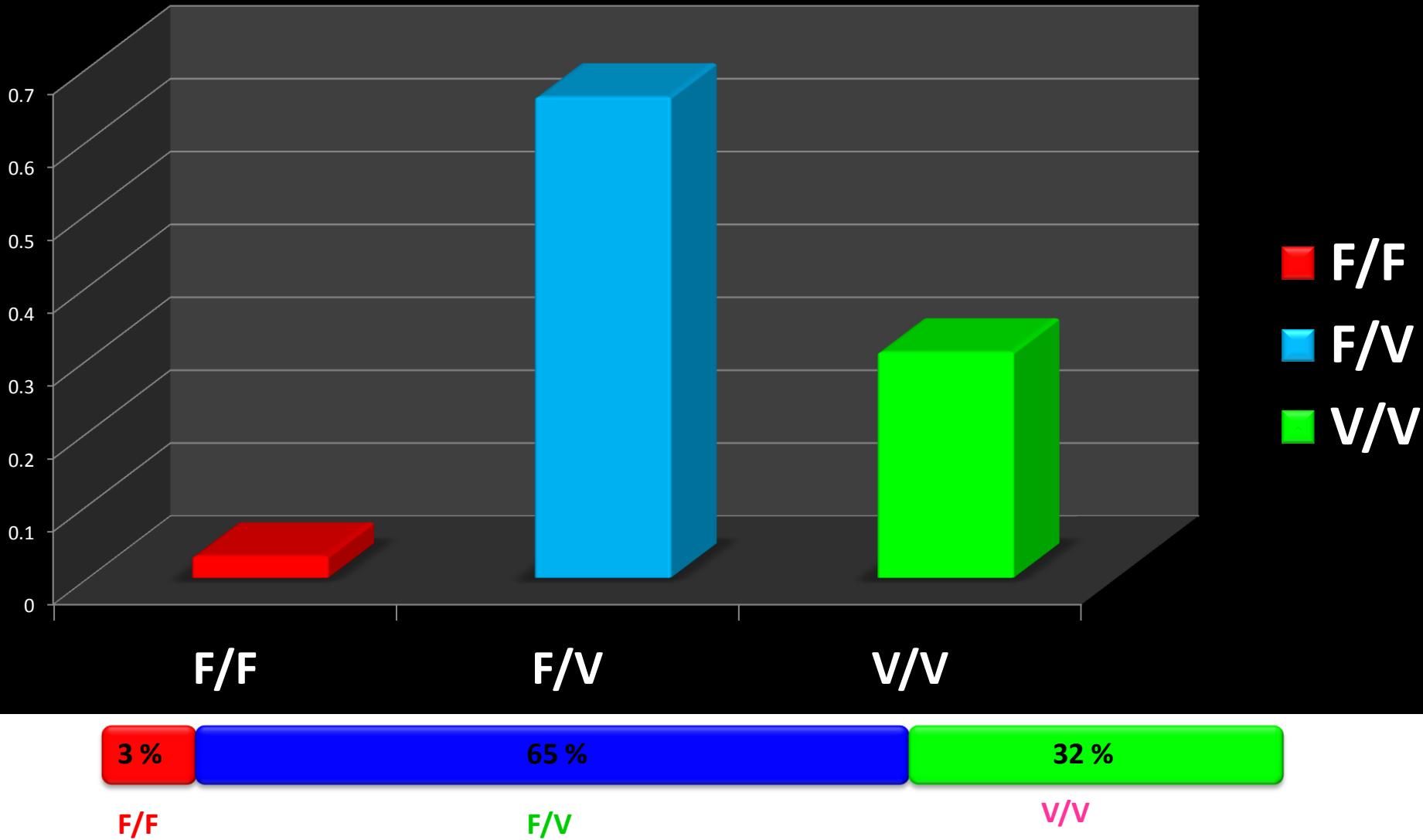


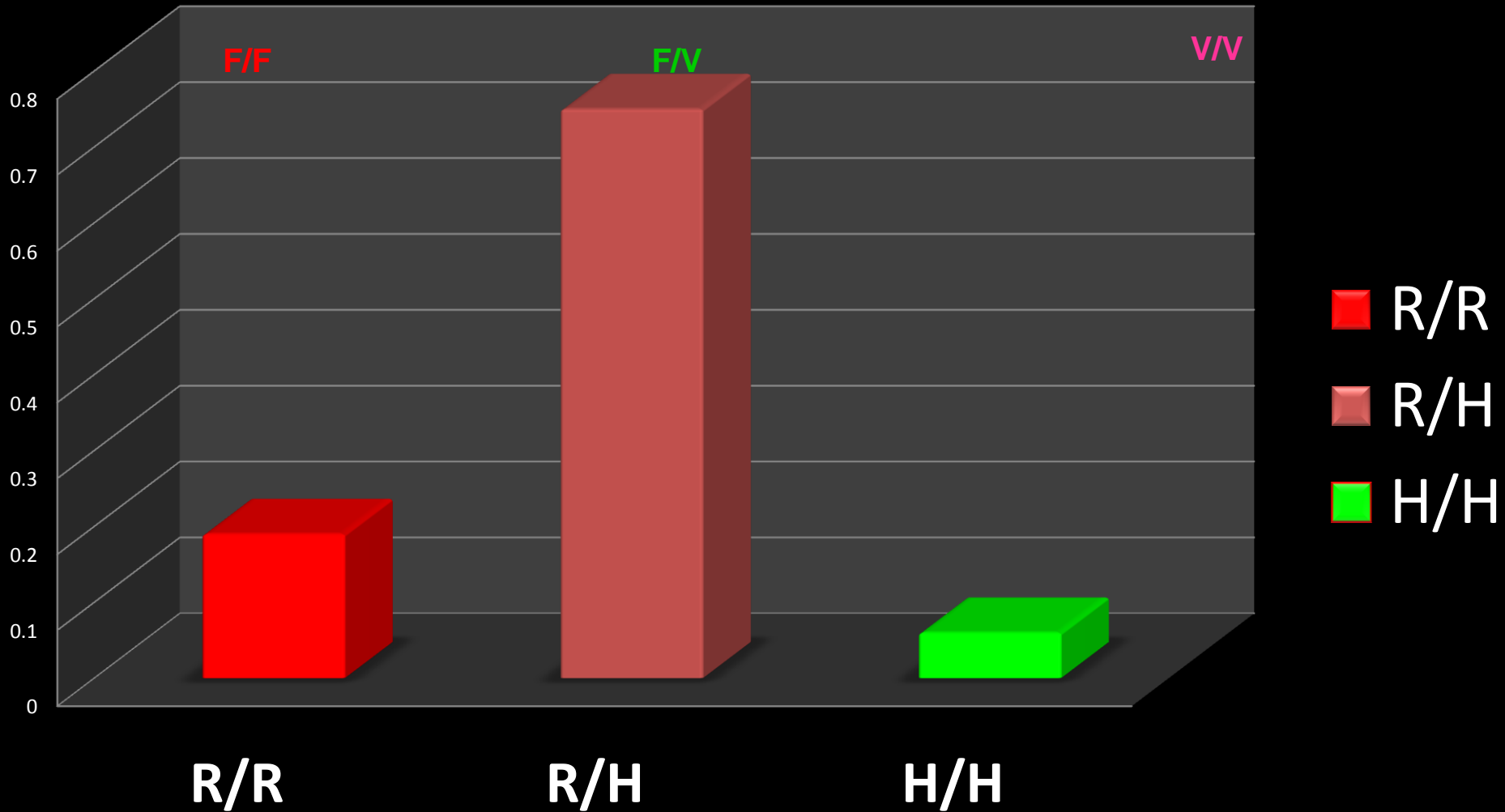
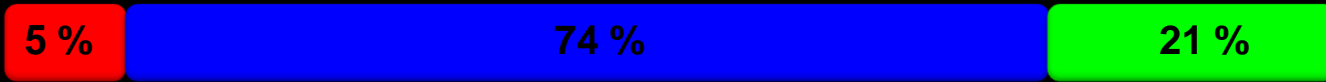
Barok M et al. Mol Cancer Ther 2007;6:2065-2072

In vitro trastuzumab-mediated ADCC against ErbB2-positive tumor cells.










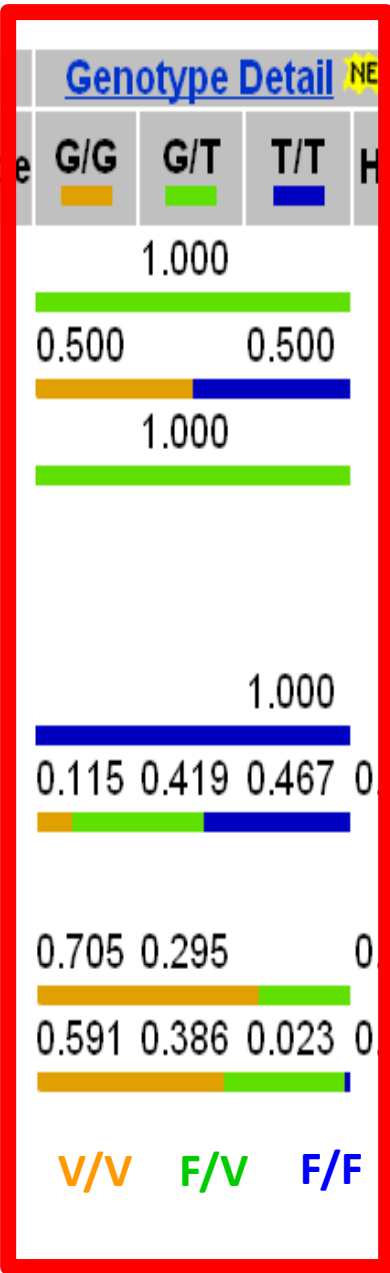
Barok M et al. Mol Cancer Ther 2007;6:2065-2072





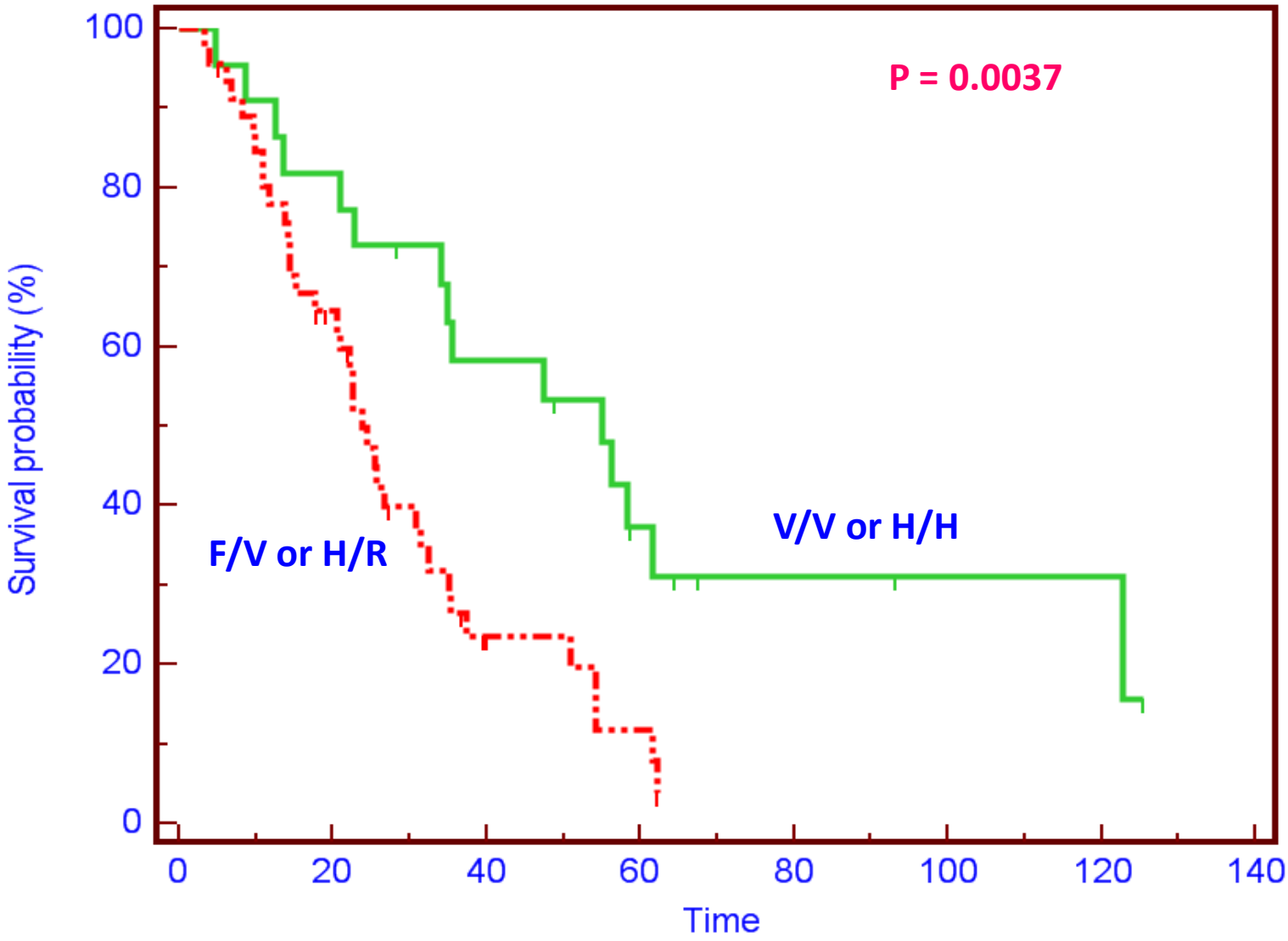
Population Diversity

		Sample Ascertainment				Genotype Detail <small>NEW</small>				Alleles	
ss#	Population	Individual Group	Chrom. Sample Cnt.	Source	G/G 	G/T 	T/T 	HNP	G 	T 	
ss139195483	ENSEMBL_Venter		2	IG	1.000 				0.500	0.500	
	ENSEMBL_celera		4	IG	0.500		0.500		0.500	0.500	
ss165307845	CEU	European	2	IG	1.000 				0.500	0.500	
ss218676042	pilot_1_YRI_low_coverage_panel		118	AF					0.042	0.958	
ss230749579	pilot_1_CEU_low_coverage_panel		120	AF					0.117	0.883	
ss3176083	MITOGPOP6	multiple	14	IG			1.000			1.000	
ss342007110	ESP_Cohort_Populations		4410	GF	0.115	0.419	0.467	0.050	0.324	0.676	
ss515482	CEPH		184	AF					0.450	0.550	
	HapMap-HCB	Asian	88	IG	0.705	0.295		0.584	0.852	0.148	
	HapMap-JPT	Asian	88	IG	0.591	0.386	0.023	0.871	0.784	0.216	



Summary	Average Het. +/- std err:	Individual Count	Founders Count	Individual Overlap	Genotype Conflict
	0.450 +/- 0.150	186	183	0	0

REM



**Over All
Survival (OS)**

Number at risk

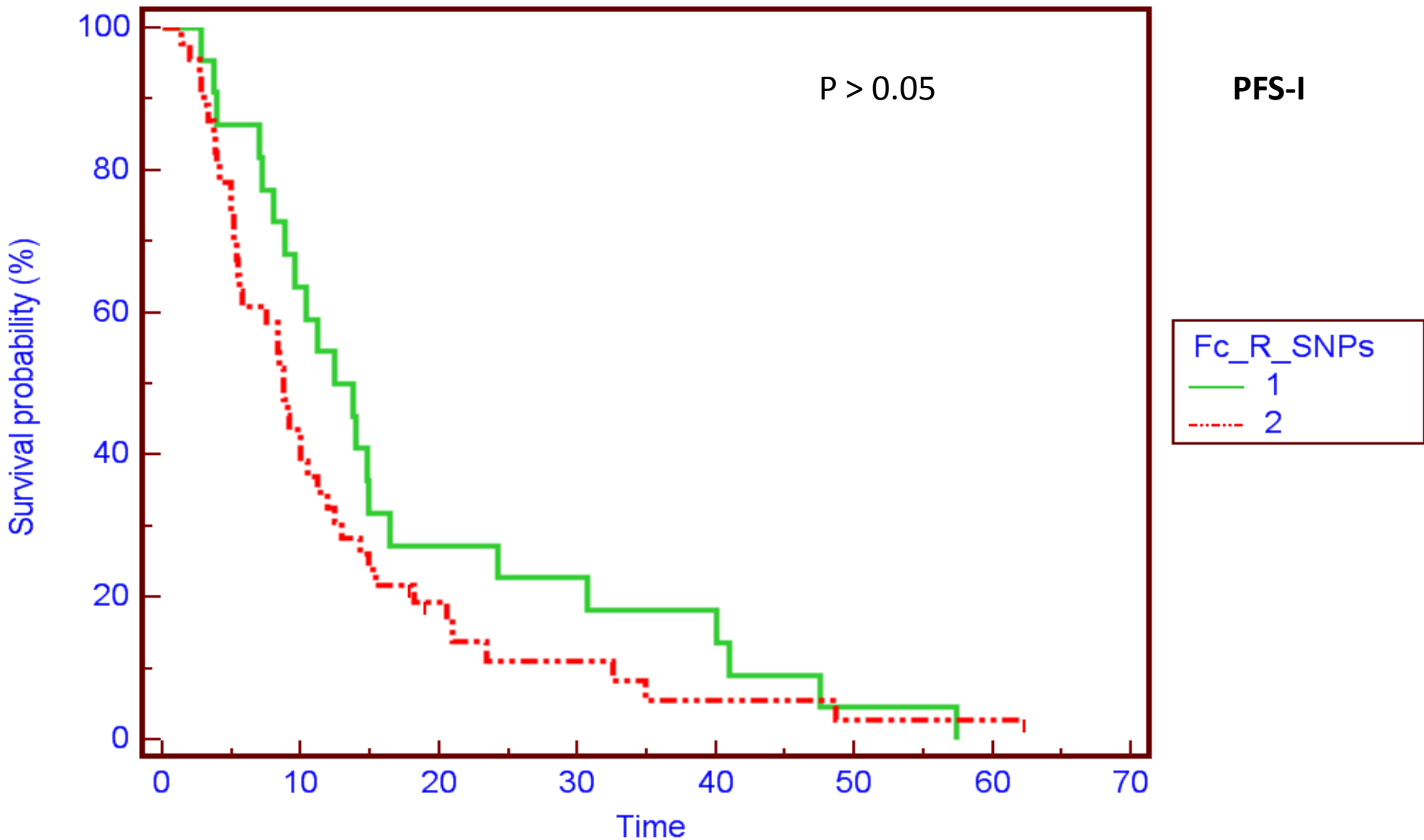
Group: 1

22 18 12 6 3 2 2 1

Group: 2

46 27 7 3 0 0 0 0

REM1



Number at risk

Group: 1

22	14	6	5	3	1	0	0
----	----	---	---	---	---	---	---

Group: 2

46	18	7	4	2	1	1	1
----	----	---	---	---	---	---	---

GeneView via analysis of contig annotation: [FCGR3A](#) *Fc fragment of IgG, low affinity IIIa, receptor (CD16a)*

▼ View more variation on this gene (click to hide).

Clinical Source: in gene region cSNP has frequency double hit

Primary Assembly Mapping

Assembly	SNP to Chr	Chr	Chr position	Contig	Contig position	Allele
GRCh37.p2	-	1	161514542	NT_004487.19	13003184	A

RefSeqGene Mapping

RefSeqGene	Gene (ID)	SNP to RefSeqGene	Position	Allele
NG_009066.1	FCGR3A (2214)	+	10872	T

Gene Model(s)

Function	mRNA				Protein		
	SNP to mRNA	Accession	Position	Allele change	Accession	Position	Residue change
missense	+	NM_000569.6	818	TTT ⇒ GTT	NP_000560.5	212	F [Phe] ⇒ V [Val]
missense	+	NM_001127592.1	815	TTT ⇒ GTT	NP_001121064.1	211	F [Phe] ⇒ V [Val]
missense	+	NM_001127593.1	681	TTT ⇒ GTT	NP_001121065.1	176	F [Phe] ⇒ V [Val]
missense	+	NM_001127595.1	663	TTT ⇒ GTT	NP_001121067.1	176	F [Phe] ⇒ V [Val]
missense	+	NM_001127596.1	660	TTT ⇒ GTT	NP_001121068.1	175	F [Phe] ⇒ V [Val]



PIK3CA/Akt Pathway and FcγRIIIa Impact on Over All Survival (OS)

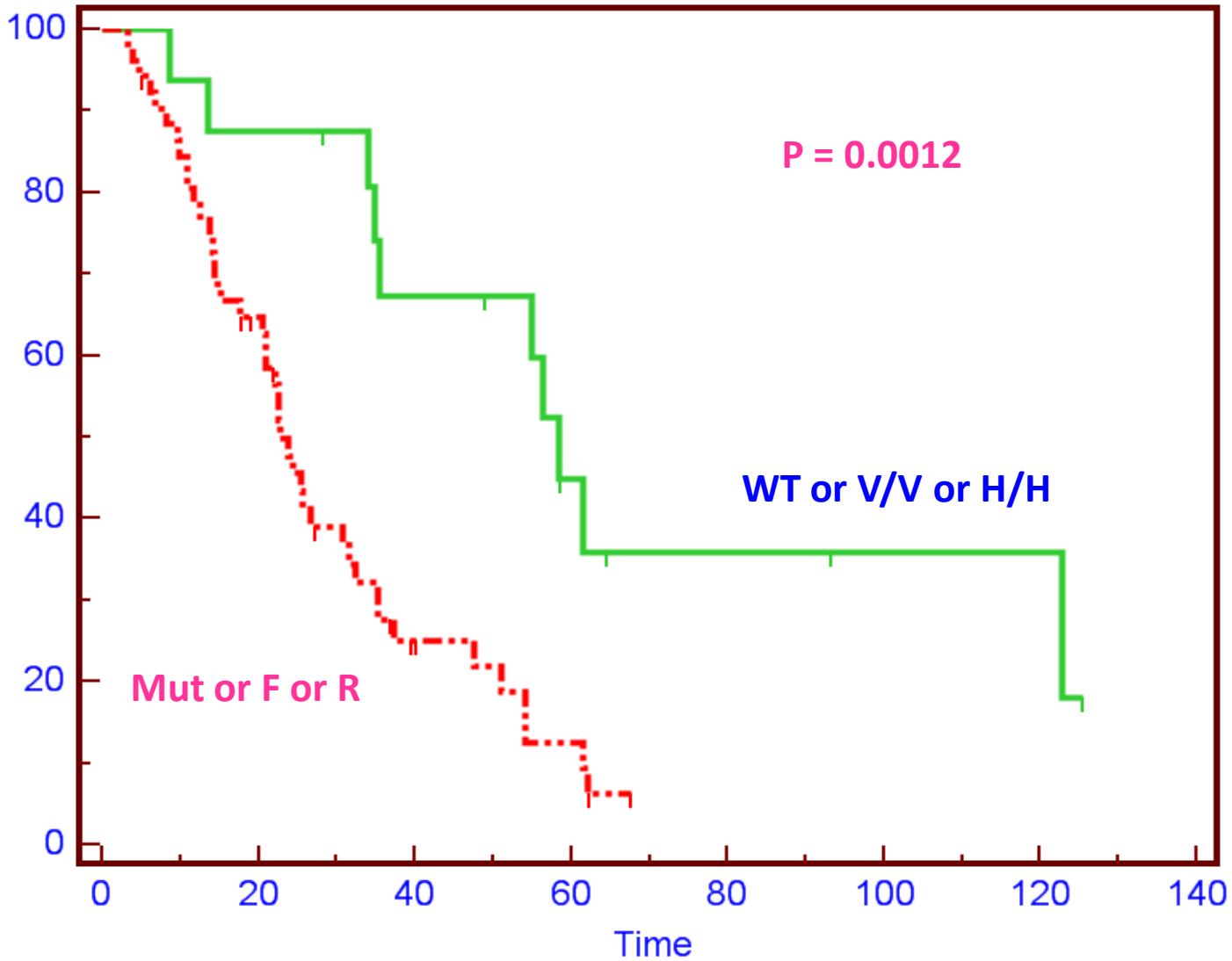
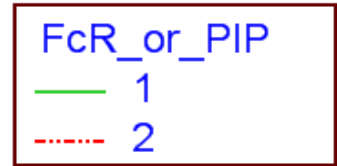
REM

OS

P = 0.0012

WT or V/V or H/H

Mut or F or R



Number at risk

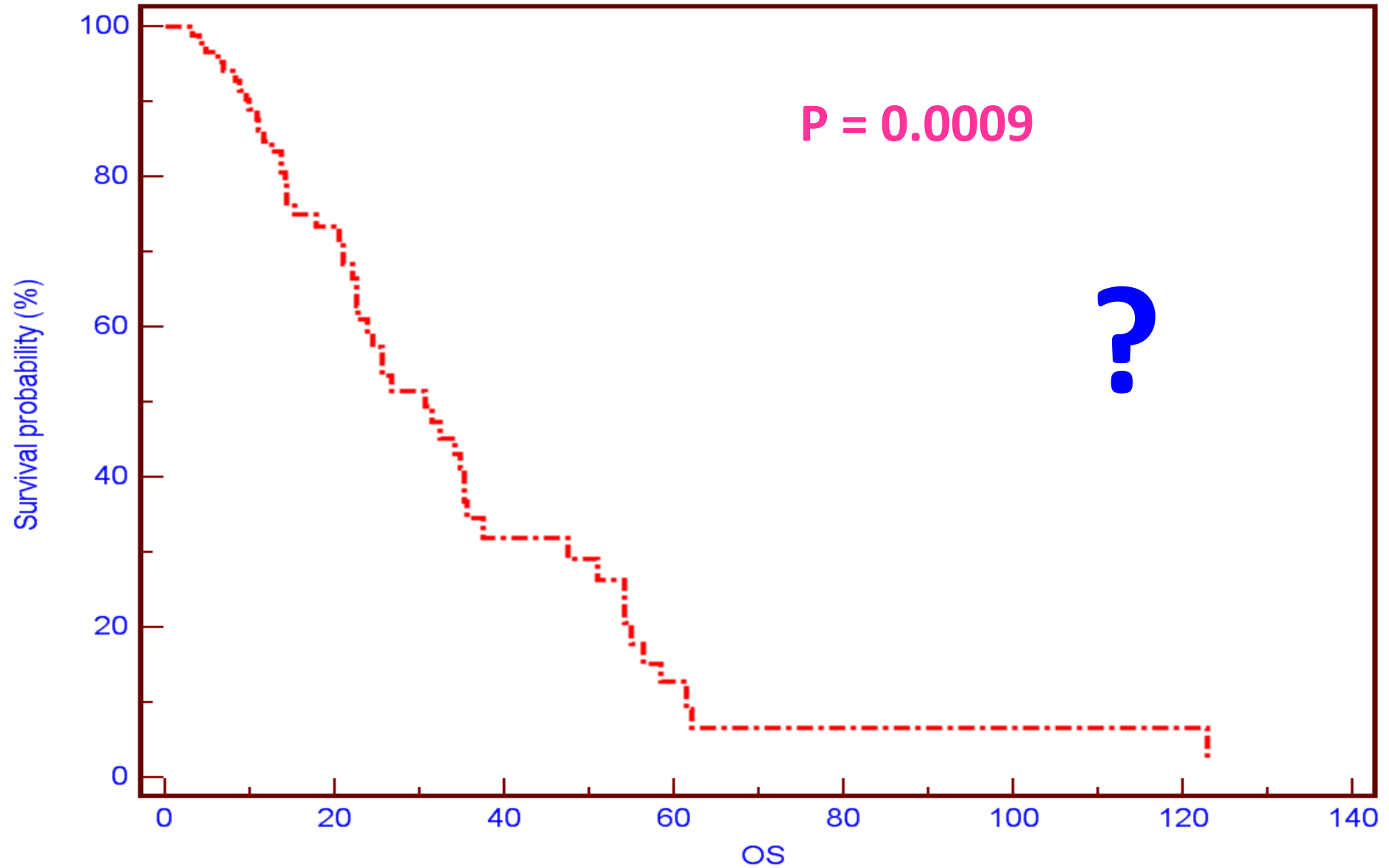
Group: 1

16 14 10 5 3 2 2 1

Group: 2

52 31 9 4 0 0 0 0

Survival at mean of covariates

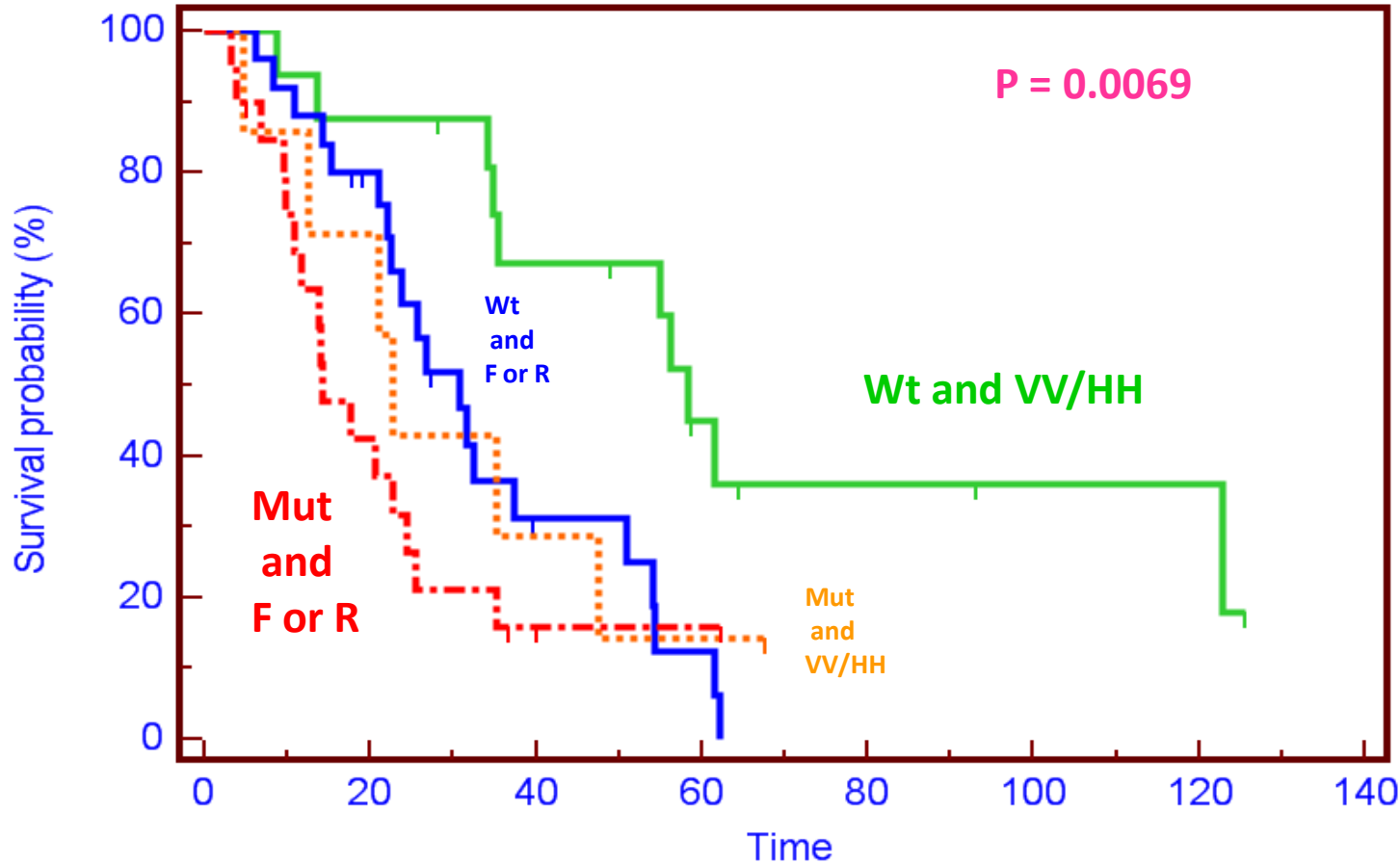


COX proportional analysis of PIK3CA/Akt1 pathway and FcgR SNPS

REM

OS

P = 0.0069



Number at risk

Group: 1

16 14 10 5 3 2 2 1

Group: 2

25 18 5 2 0 0 0 0

Group: 3

7 5 2 1 0 0 0 0

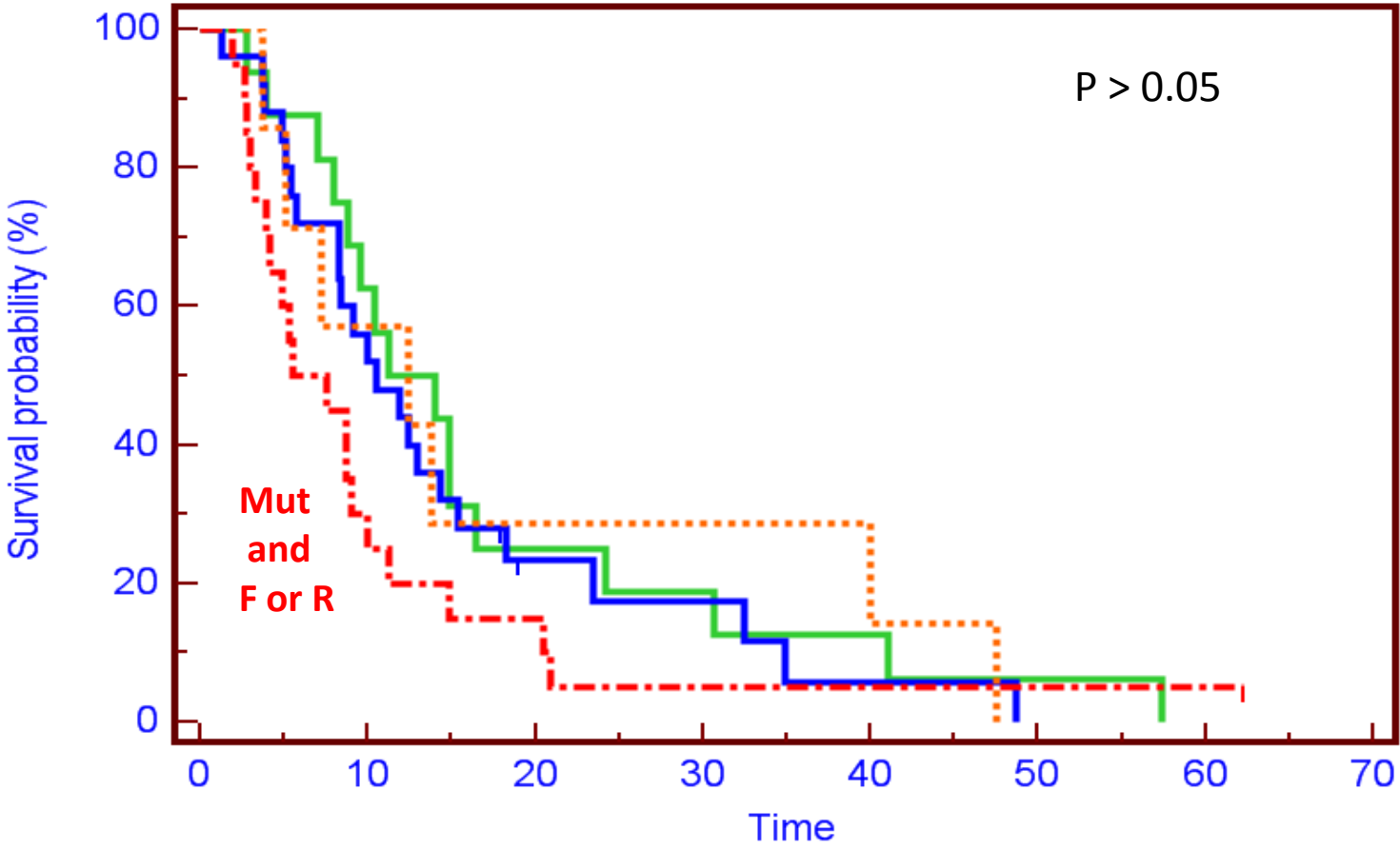
Group: 4

20 8 2 1 0 0 0 0

REM1

PFS-I

P > 0.05



Number at risk

Time	0	10	20	30	40	50	60	70
Group: 1	16	10	4	3	2	1	0	0
Group: 2	25	13	4	3	1	0	0	0
Group: 3	7	4	2	2	1	0	0	0
Group: 4	20	5	3	1	1	1	1	1

MY CANCER GENOME

Breast Cancer Overview

- ▼ AKT1
- ▼ PIK3CA
- ▼ PTEN

Other Cancers

- ▶ Molecular Pathology
- ▶ Take Our Survey
- ▶ Glossary
- ▶ News
- ▶ Our Team
- ▶ Acknowledgments

Molecular Profiling of Breast Cancer

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 (Jemal, 2010; Jemal, 2011). Classically, treatment decisions have been based upon histology of the tumor and on the status of three main biomarkers: estrogen receptor (ER), progesterone receptor (PR), and HER2/neu. Despite significant improvements in the treatment of breast cancer, novel therapies and treatment strategies are needed.

Molecular alterations involving the PI3K/AKT pathway (Figure 1) occur in over 30% of invasive breast tumors. Alterations in breast cancer resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in *PIK3CA* (the gene encoding the PI3K catalytic subunit p110 α), mutations in *AKT1*, amplification of *AKT2*, and loss of the phosphatase *PTEN* (Engelman, 2006). Mutations in *PIK3CA* cluster in two major 'hot spots' located in the helical (E542K and E545K in exon 9) and catalytic (H1047R in exon 20) domains (Saal, 2005; Bachman, 2004). Expression of these mutant p110 α isoforms confers growth factor-independent proliferation and protection from anoikis (a form of cell death) and chemotherapy.

Both genetic and biochemical data suggest that activation of the PI3K/AKT survival pathway contributes to breast cancer development and tumorigenesis. *PIK3CA* mutations in primary breast tumors have been associated with lymph node metastases, presence of ER and PR, and HER2 overexpression (Saal, 2005; Stemke-Hale, 2008). The impact of these mutations and of *PTEN* loss on the virulence of breast cancer and patient outcome is not completely clear yet. However, PI3K hyperactivity has been associated with resistance to anti-HER2 and anti-estrogen therapies. For example, presence of activating *PIK3CA* mutations and loss of *PTEN* in HER2-overexpressing cancers correlates with a lower response to trastuzumab and lapatinib (Berns, 2007; Nagata, 2004; Serra, 2008). Prospective studies to confirm these

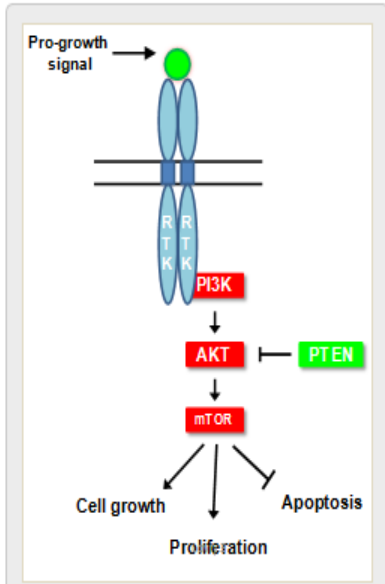
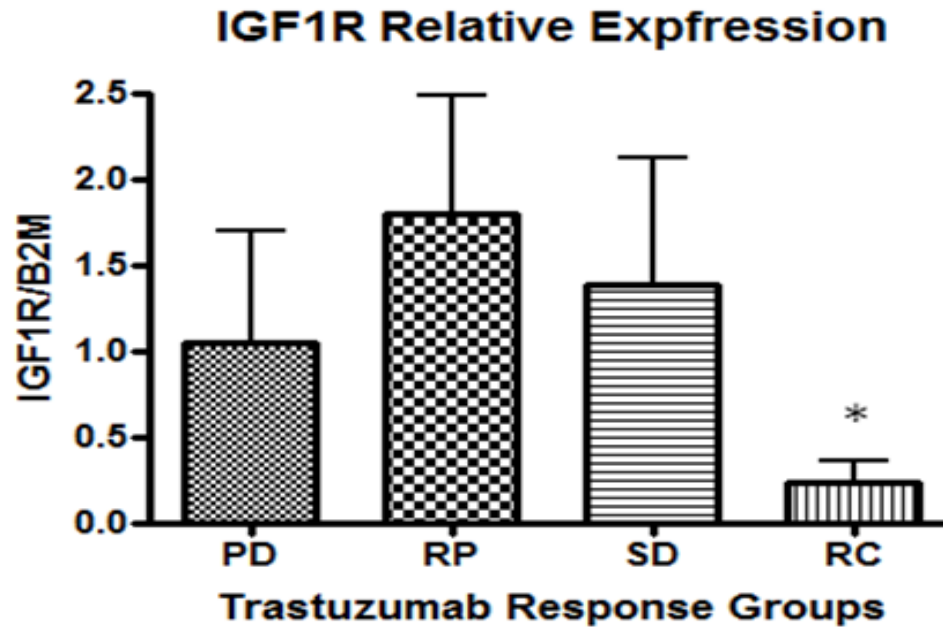


Figure 1: Schematic of the PI3K/AKT signaling pathway. Growth factor binding to receptor tyrosine kinases results in activation of the PI3K signaling pathway (PI3K-AKT-mTOR). The letter "K" within the schema denotes the tyrosine kinase domain.



IGF-R1 expression



If I did something perfect, it's because I have the following team, otherwise it was my fault.....



