Characterization of primary and immune escape variant of HER-2/neu over-expressing mouse mammary carcinoma

Masoud H. Manjili

Department of Microbiology & Immunology
Virginia Commonwealth University, Richmond VA

iSBTc November 12, 2005

Tumor Relapse

Treatments for metastatic tumors:

- Radiation
  - Radiation-resistant tumors develop

  relapse
- Chemotherapy
  - Drug-resistant tumors develop

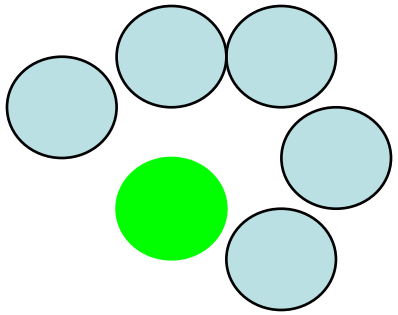
  relapse
- Immunotherapy
  - Tumor escape

  relapse

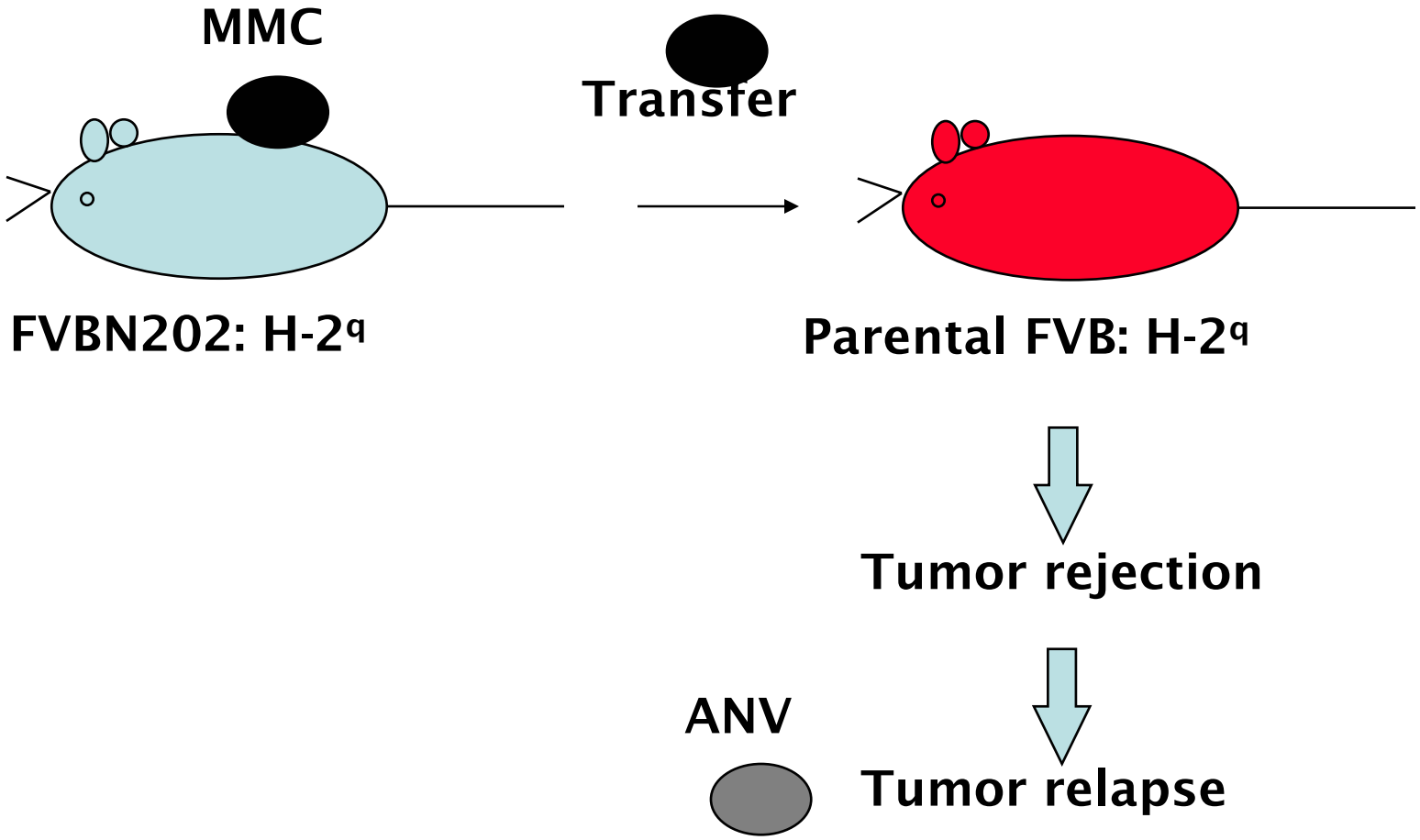
Immunotherapy may select for relapse phenotype of tumors: antigen loss

1. Epigenetic changes?

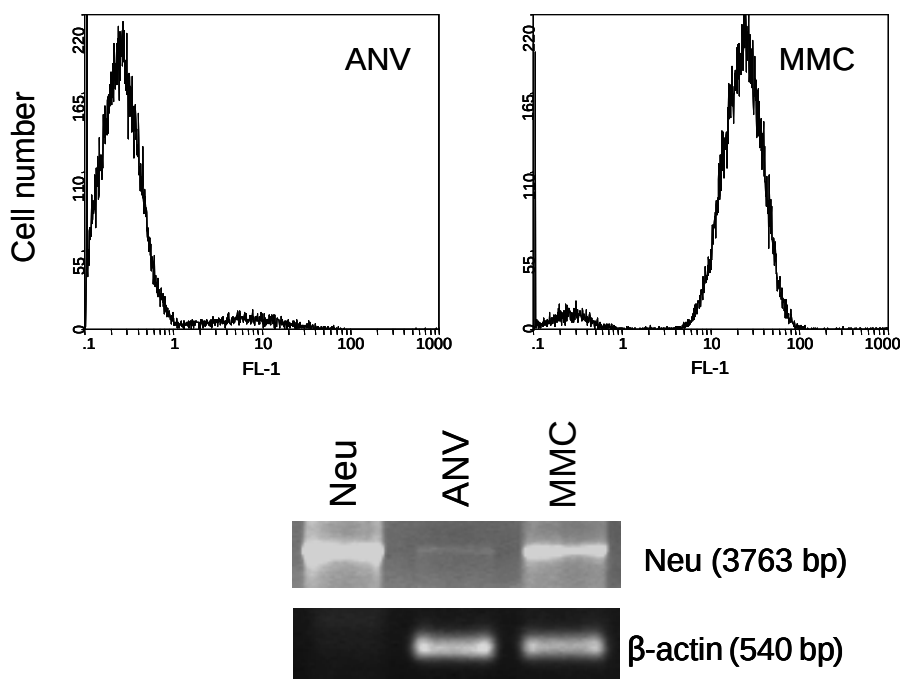
2. Darwinian selection?

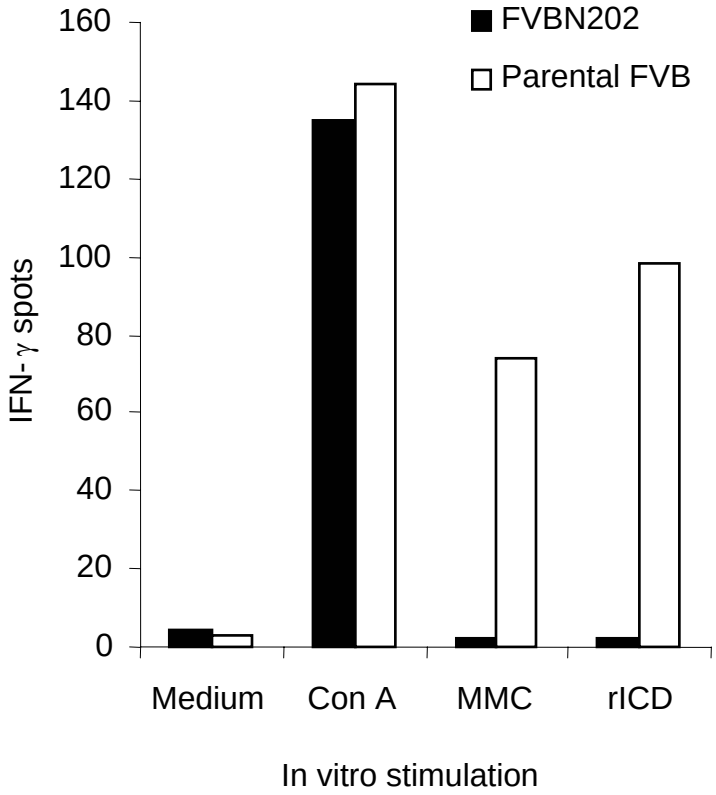


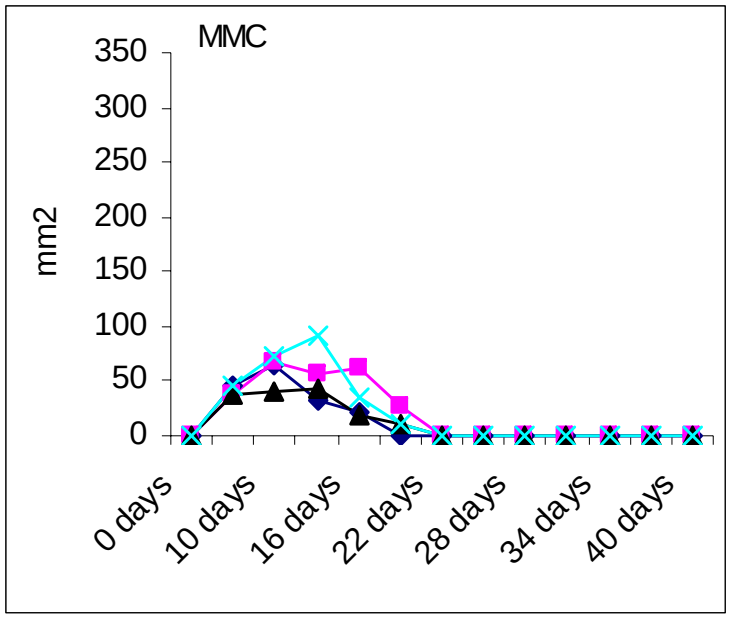
Emergence of relapse phenotype from primary tumors

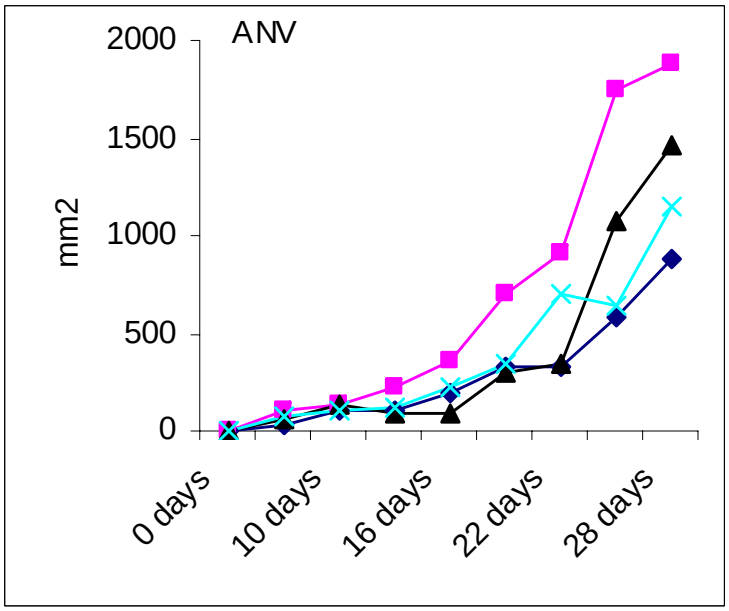


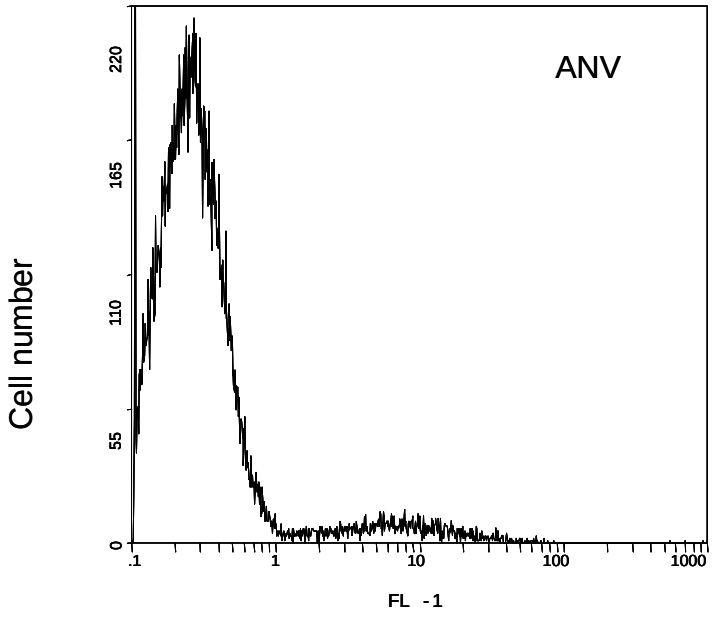
Tumor relapse in the presence of neu-specific immune responses

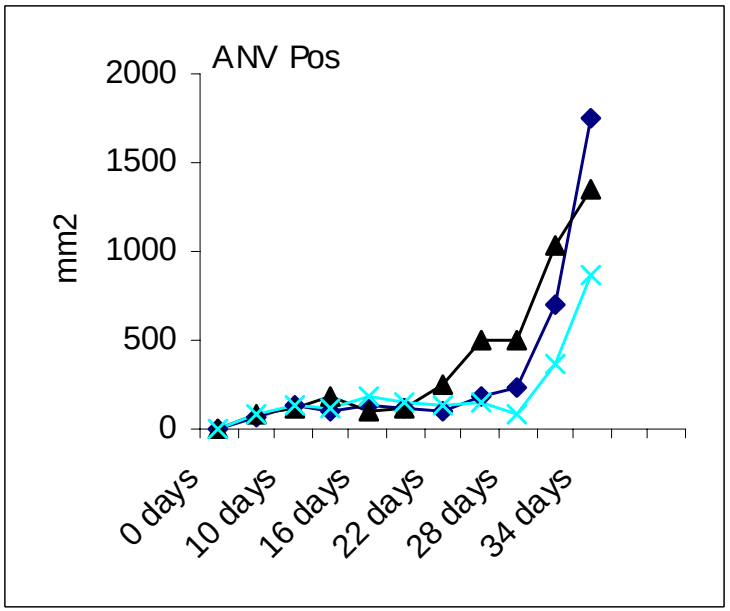




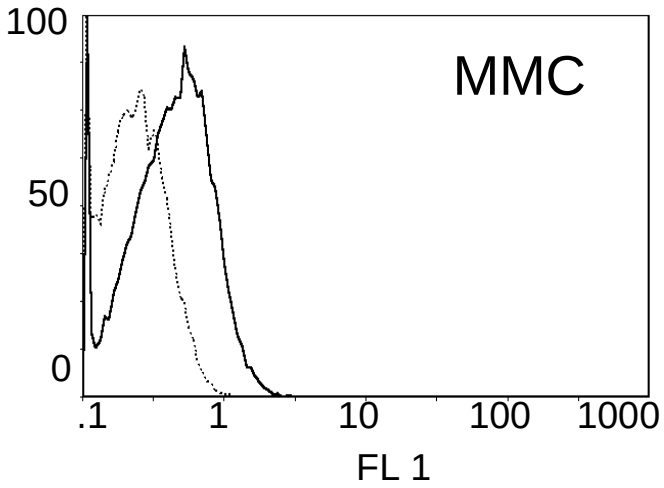

MMC
mm2
350
300
250
200
150
100
50
0
0 days
10 days
16 days
22 days
28 days
34 days
40 days

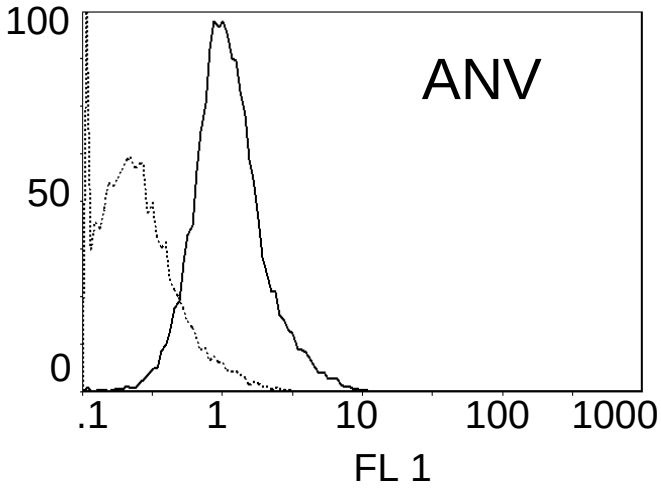

ANV
mm2
2000
1500
1000
500
0
0 days
10 days
16 days
22 days
28 days

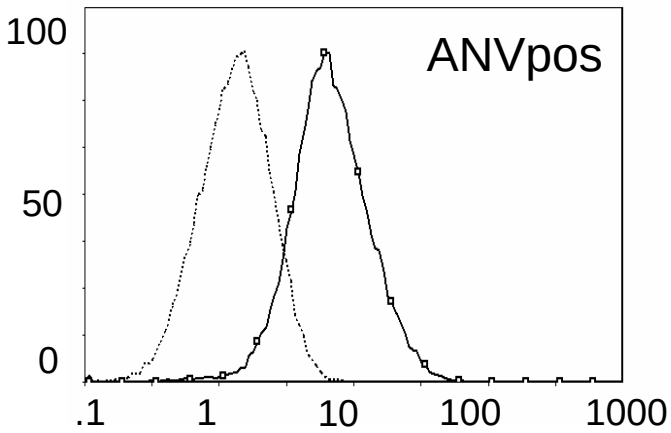

ANV
Cell number
220
165
110
55
0
.1
1
10
100
1000
FL -1


ANV Pos
mm2
2000
1500
1000
500
0
0 days
10 days
16 days
22 days
28 days
34 days

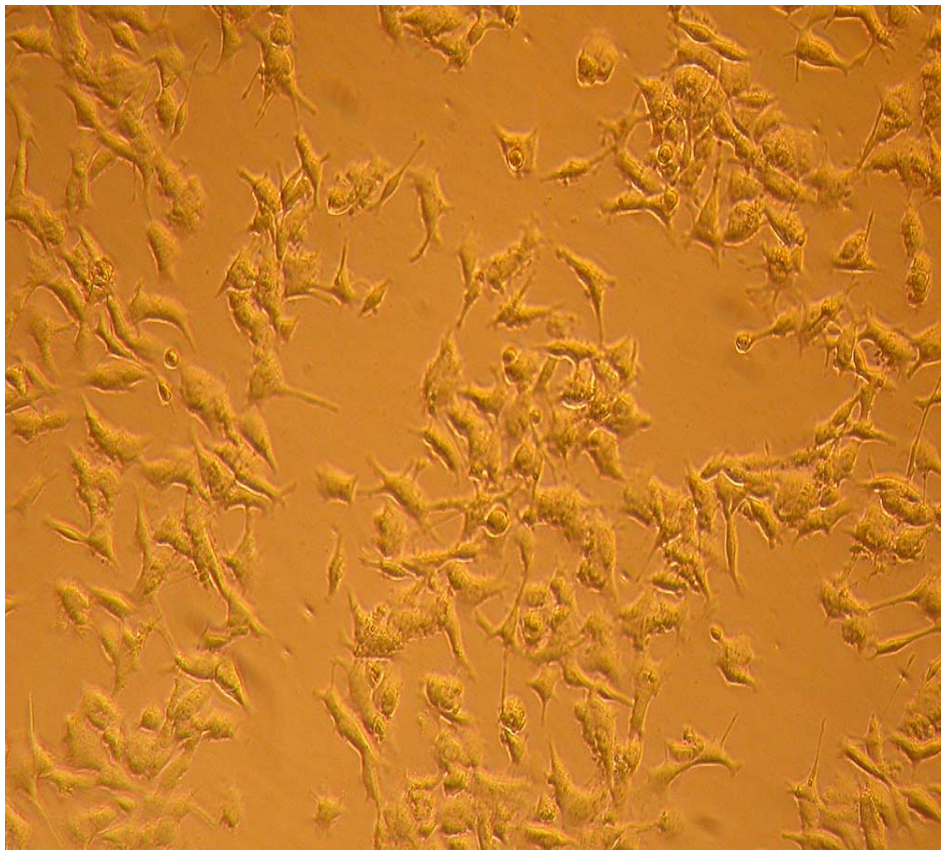
Higher expression of H-2D^q/H-2L^q in ANV



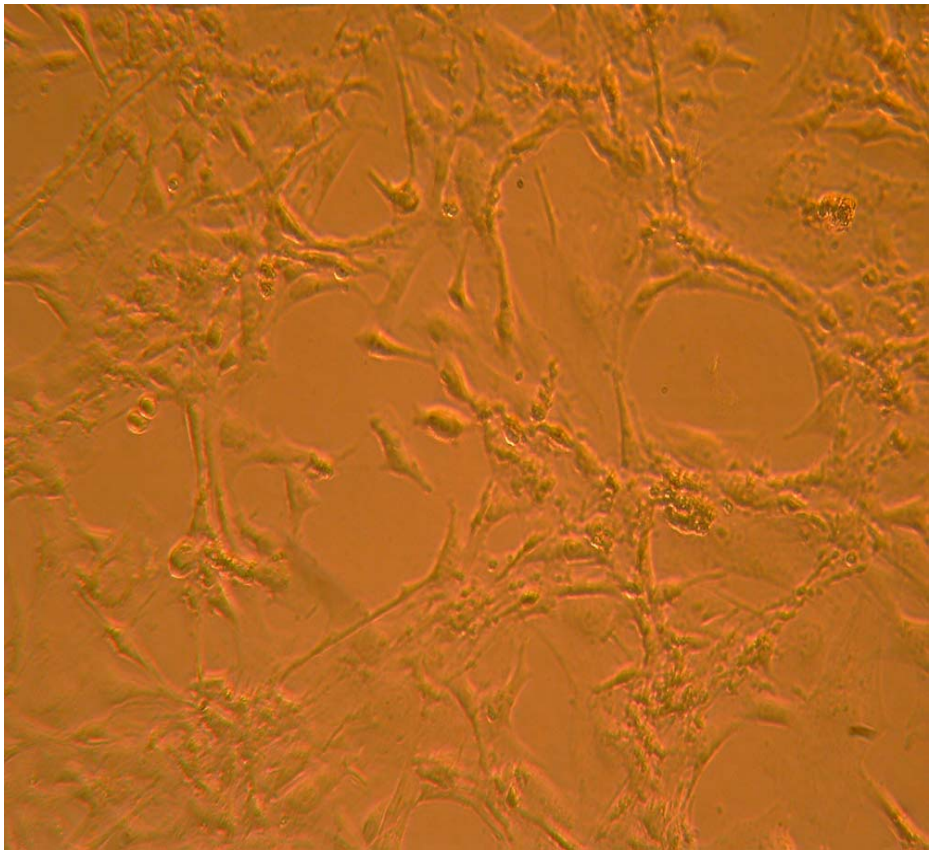




MMC

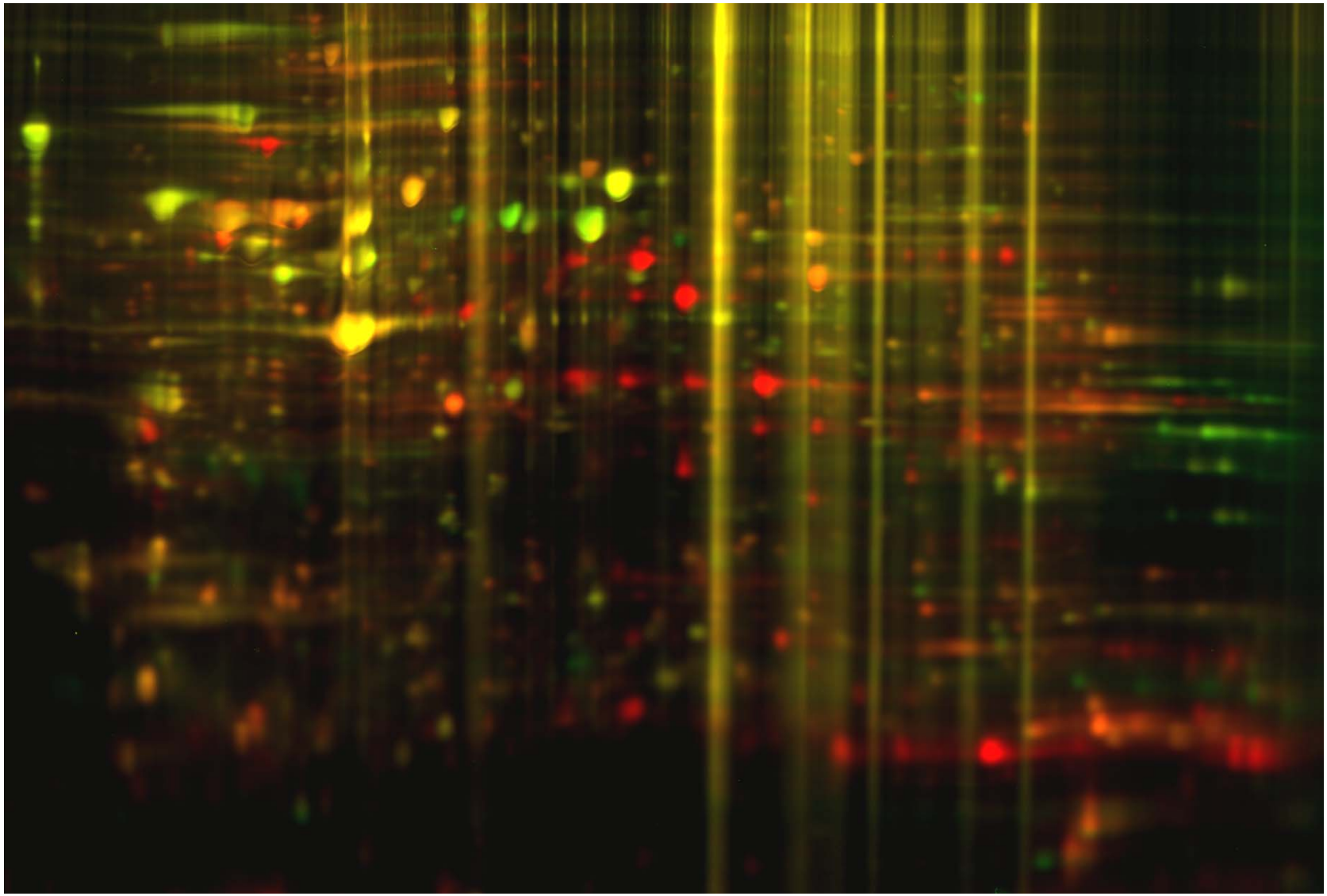


ANV



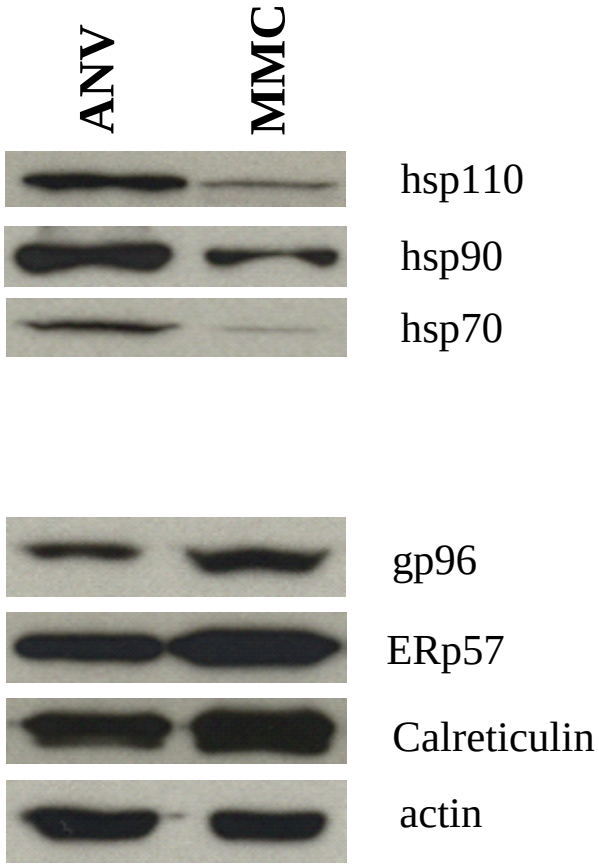
Cy3 : MMC

Cy5 : ANV



Spot #	Protein ID	Accession #	Score [a]	Peptides matched	MW Kd	IP	Coverage %	Fold Difference
1	Rho guanine nucleotide exchange factor (GEF7)	NP_059098	67	13	73	6.85	20	73.15
2	Myosin (Myh9)	AAH44834	121	11	155	5.43	12	25.23
3	Hnrp A3 protein	AAH62198	96	9	34	9.08	28	5.21
4	Retinol binding protein 1	NP_035384	113	12	16	5.1	71	4.7
5	Annexin A1	AAH02289	179	12	38	6.97	40	4.37
6	Cortactin	NP_031829	68	5	61	5.24	9	4.26
7	Enolase1	AAH39179	106	9	50	7.67	26	4.11
8	Oat protein	AAH08119	90	7	48	6.19	18	4.04
9	Annexin A3	NP_038498	97	8	36	5.33	30	3.99
10	Peroxiredoxin 6	AAP211829	69	5	24	5.71	25	3.99
11	Tubulin,beta	CAE84031	214	21	50	4.78	50	3.88
12	Hnrp A1 protein	AAH80675	74	6	34	9.27	25	3.76
13	Peroxiredoxin 1	NP_035164	92	12	22	8.26	53	3.65
14	Vimentin	CAA69019	171	13	51	4.96	34	3.45
15	Phosphoglycerate mutase 1	AAH02241	146	6	28	6.67	38	3.38
16	Cytokeratin 8	P11679	180	17	54	5.5	33	-8.51
17	D-lactate dehydrogenase	NP_081846	119	10	52	6.15	21	-7.04
18	Transferrin	AAL34533	137	12	78	6.92	19	-5.21
19	Malate dehydrogenase	AAA39509	99	7	36	8.93	26	-5.15
20	Cytokeratin 8	AAA37551	224	22	53	5.42	41	-4.84
21	Hnrp A2/B1	NP_872591	119	7	32	8.74	31	-4.41
22	Cytokeratin 8	BAA14375	141	25	54	5.7	43	-4.66
23	GRP58 (ERp57)	AAH33439	260	17	57	5.88	38	-3.81
24	Glucose regulated protein 58	AAH33439	191	13	57	5.88	29	-3.63
25	GP96	AAH10445	88	16	92	4.74	20	-3.5
26	Tumor rejection antigen gp96	AAH10445	124	11	92	4.74	15	-3.04
27	ATP synthase	NP_058054	76	6	56	5.19	13	-3.01
28	Cytokeratin 8	AAA37551	90	7	53	5.42	17	-2.69
29	Cytokeratin 18	NP_034794	124	9	47	5.22	26	-2.66
30	Calreticulin	NP_031617	64	6	48	4.33	21	-2.51

Expression of cytosolic and ER chaperones by MMC and ANV



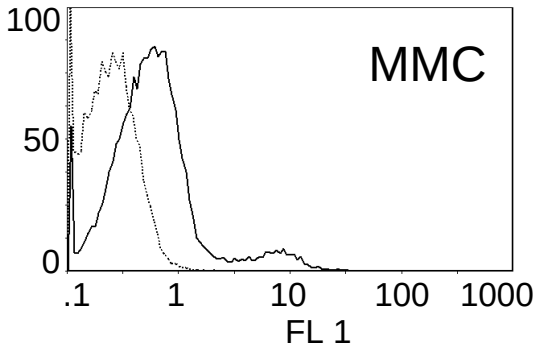
CTL mediated tumor killing

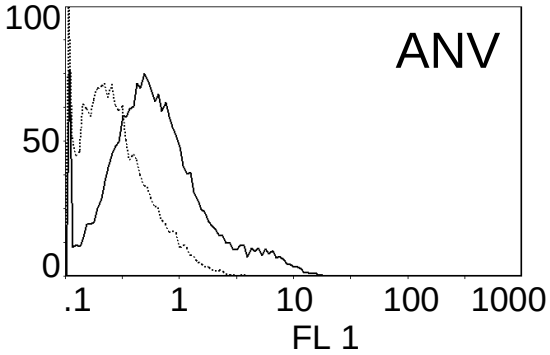
IFN-γ

Fas-Fas-L

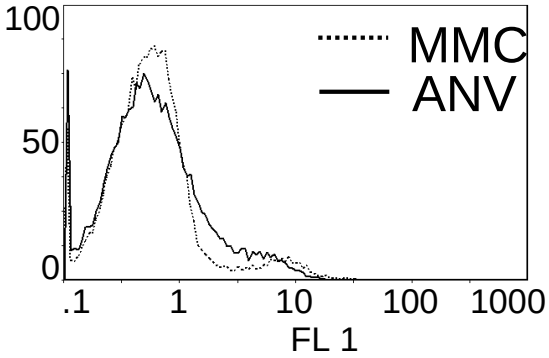
Granzyme

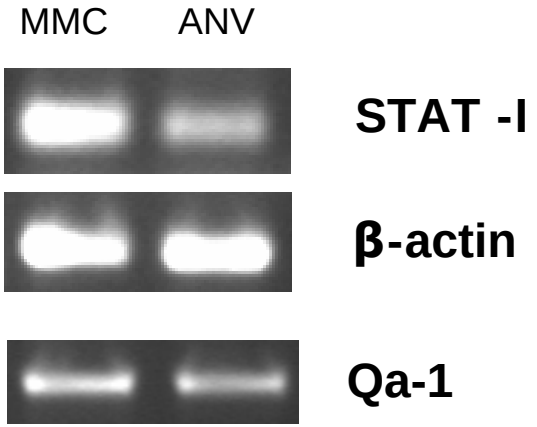
Downregulation of STAT-1 expression in ANV





IFN-γ receptor



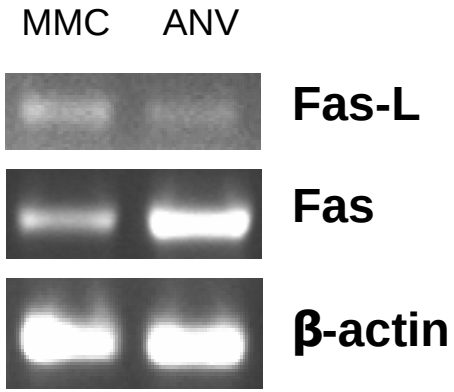


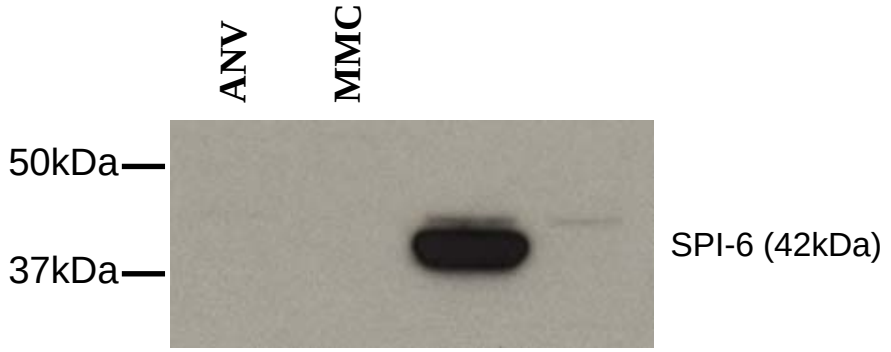
STAT -I

β-actin

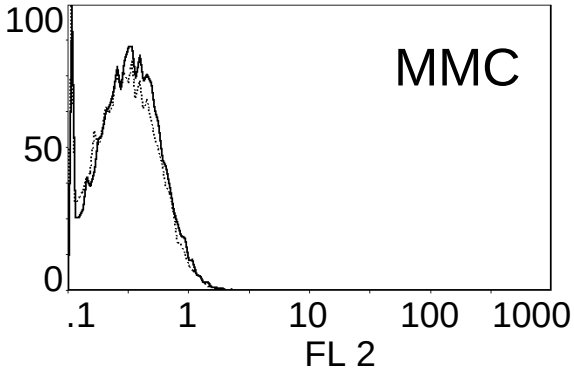
Qa-1

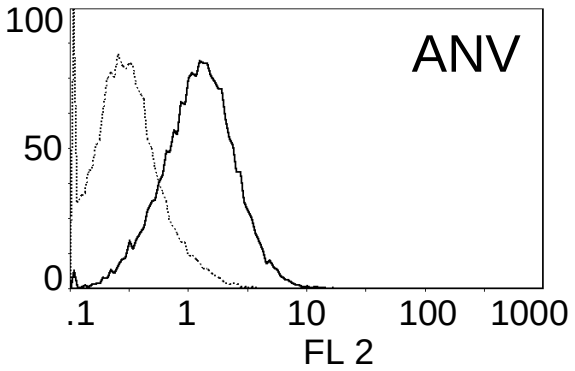
Sensitivity of ANV to Fas-mediated killing?

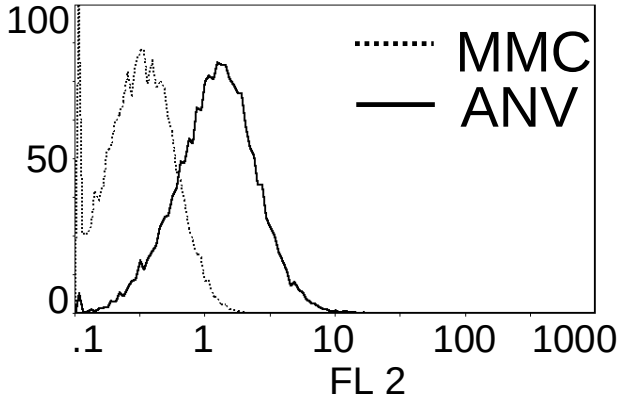




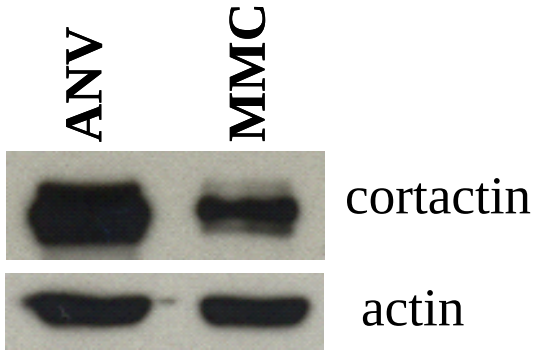
Expression of Rae-1 in ANV







Over-expression of oncogenic protein cortactin ANV over-express



Conclusion

- Neu-specific immune responses can reject primary tumors but may also induce tumor relapse through epigenetic changes in primary tumors

- Antigen loss is not a single event but only one of several other epigenetic changes that occur during tumor escape

- Cortactin could be an antigenic target to overcome tumor relapse in HER-2/neu positive mammary tumors

Acknowledgements

Manjili Lab
Maciej Kmieciak
Seema Thayil
Velusamy
Rangasamy
Hooman Nikizad

Mayo Clinic
Keith Knutson

Roswell Park Cancer Institute
John Subjeck
Latif Kazim
Hilal Arnouk

Grant supports
* R01 CA99362 (Subjeck)
* R01CA104757 (Manjili)
* Susan G. Komen BCTR0504184 (Manjili)
* Commonwealth Foundation for Cancer Research