

Presenter Disclosure Information

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The following relationships exist related to this presentation:

No relationships to disclose

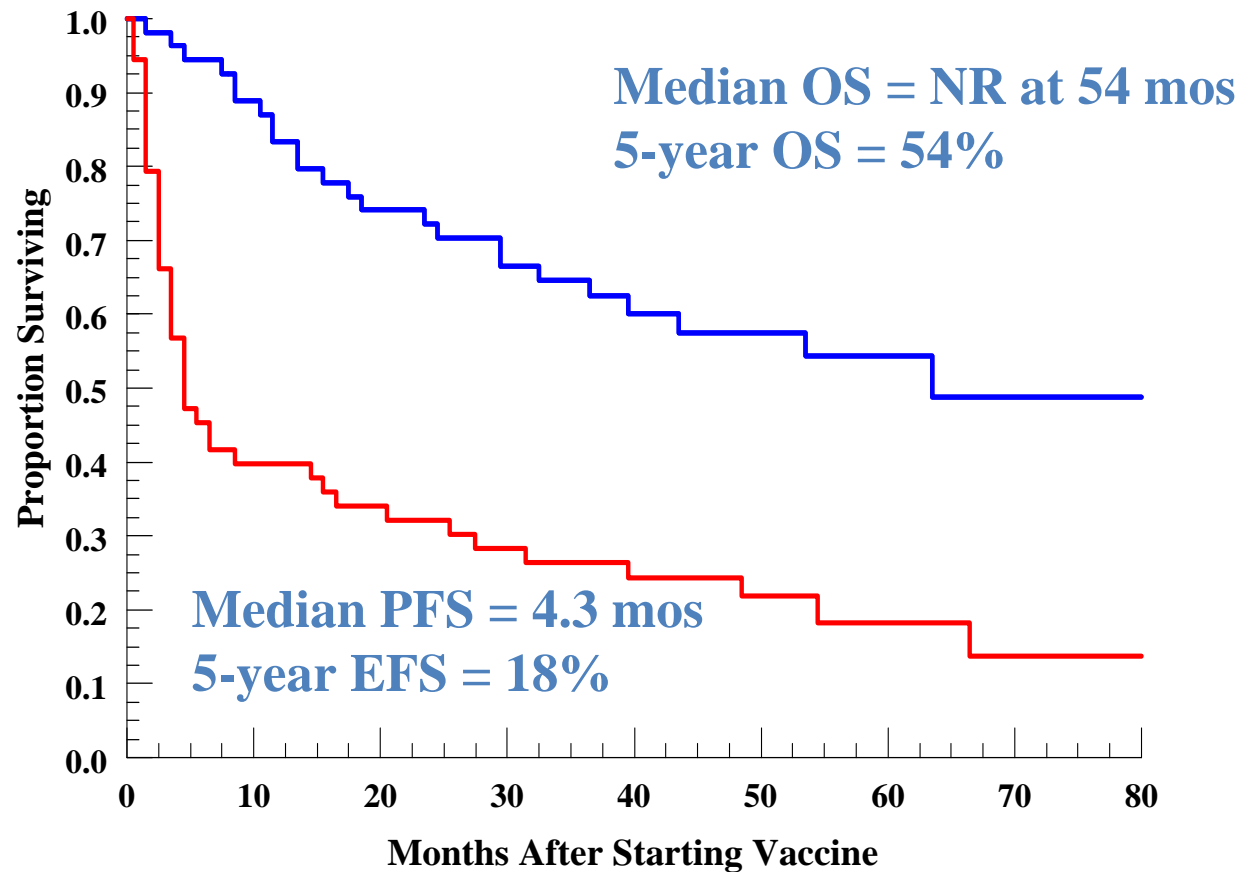
Resistance to the proapoptotic effects of IFN- γ on melanoma cells used in patient-specific dendritic cell immunotherapy is associated with improved overall survival.

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PFS and OS for metastatic melanoma patients treated with dendritic cell-tumor cell vaccine (n=54)

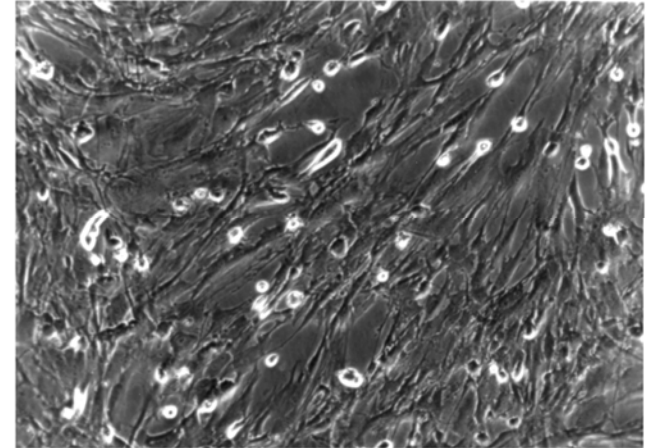


Dillman RO et al. Cancer Biother Radiopharm 24:311-319 2009



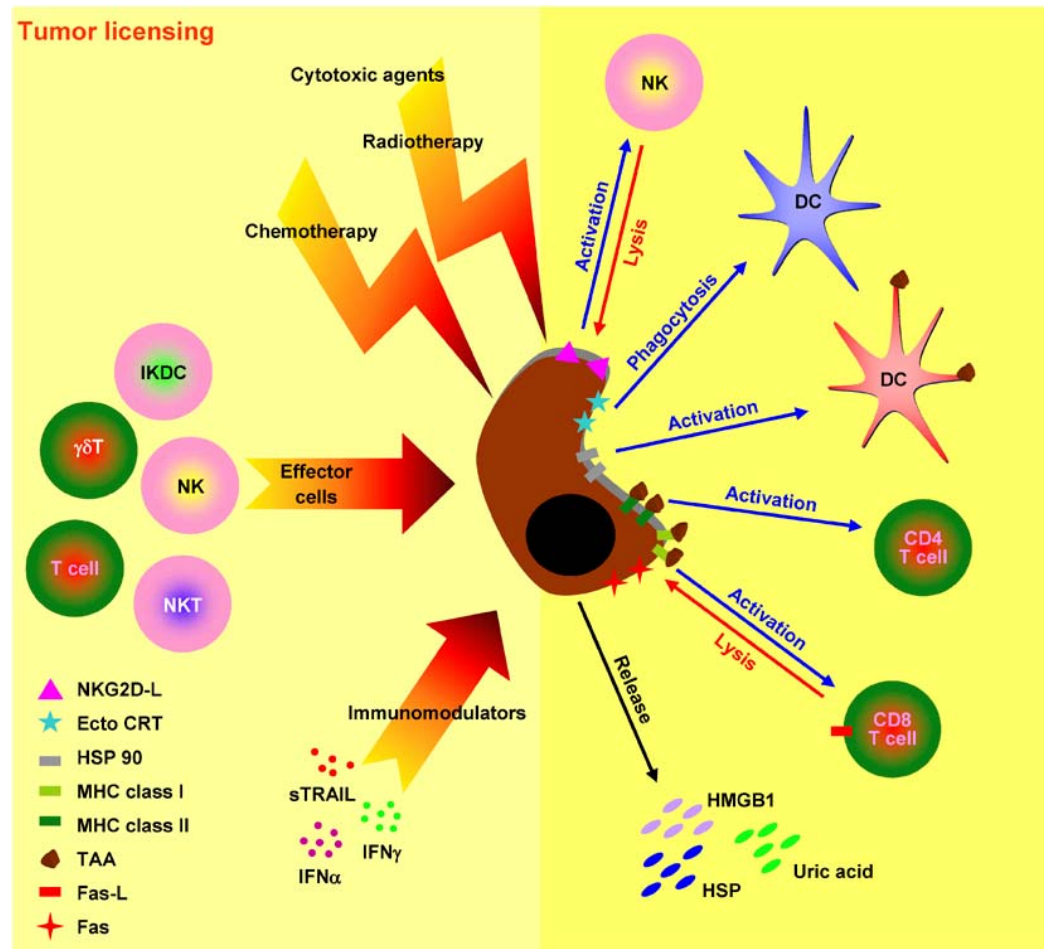
Autologous melanoma/ dendritic cell vaccine

- Autologous melanoma tumor cell lines*
 - Purified from surgical specimens
 - IFN- γ treated (1000 IU/mL for 72 hours)
 - Irradiated with 100 Gy (10,000 rad)
 - Cryopreserved
- Dendritic Cells
 - Adherent PBMCs cultured in GM-CSF/IL4 for 6 days
 - Loaded with autologous IFN- γ treated, irradiated tumor cells for 18-24 hours (DC/TC).
 - Cryopreserved
- Vaccine treatment
 - DC/TC + GM-CSF @ weeks 1, 2, 3, 8, 12, 16, 20, and 24.

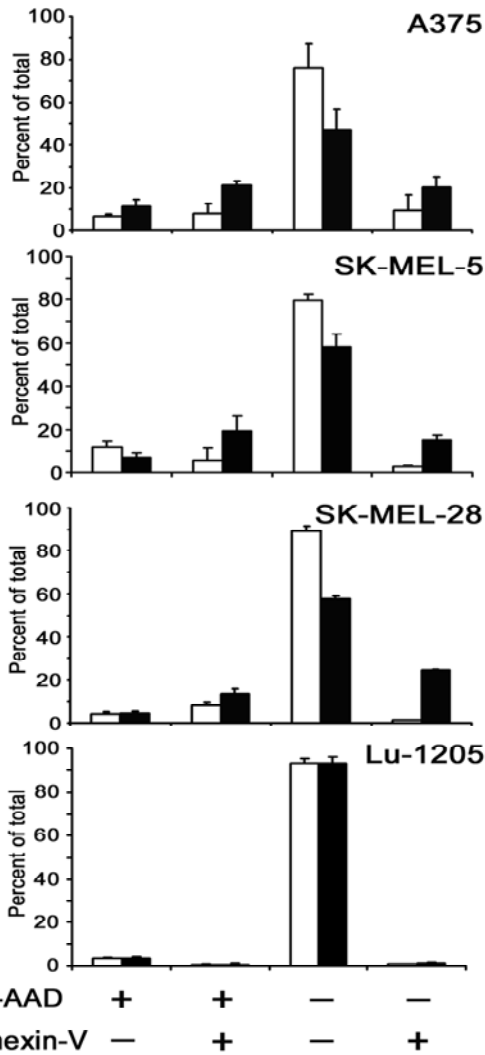
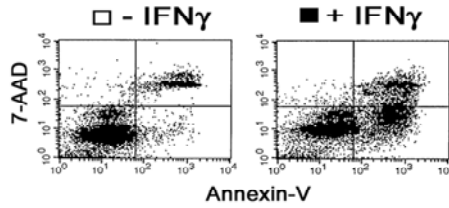


*characterized in Selvan SR et al. Melanoma Res. 20:280-92 2010

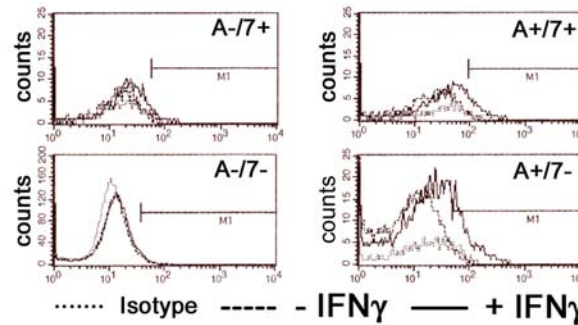
Cell surface expression of calreticulin may be regulated by IFN- γ



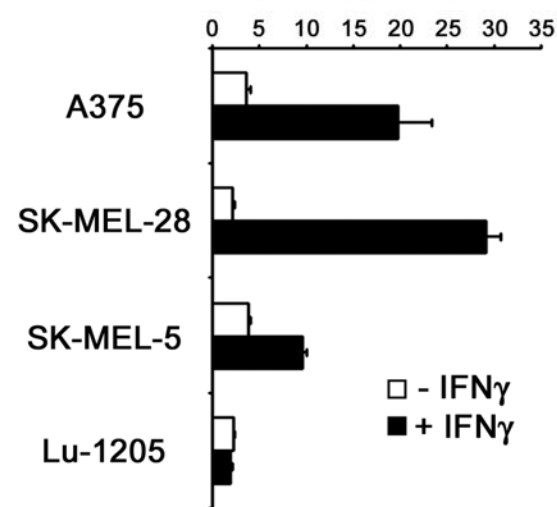
IFN- γ induces apoptosis and calreticulin exposure in melanoma cells



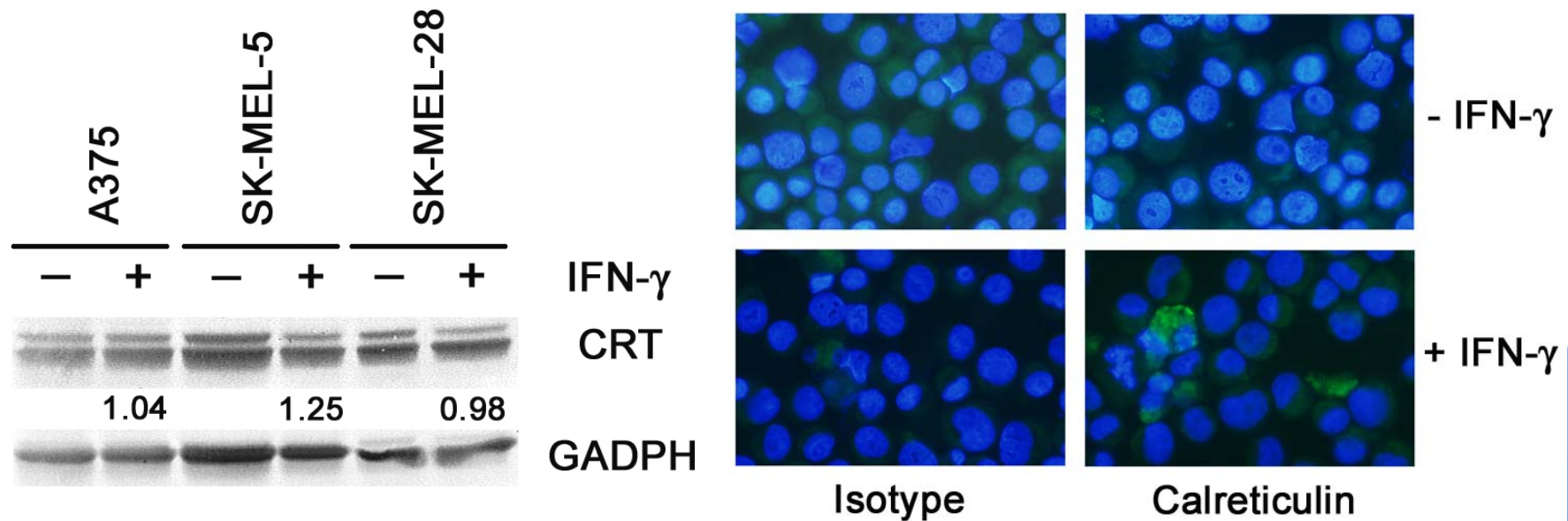
Calreticulin Expression



Percent calreticulin+/7-AAD-



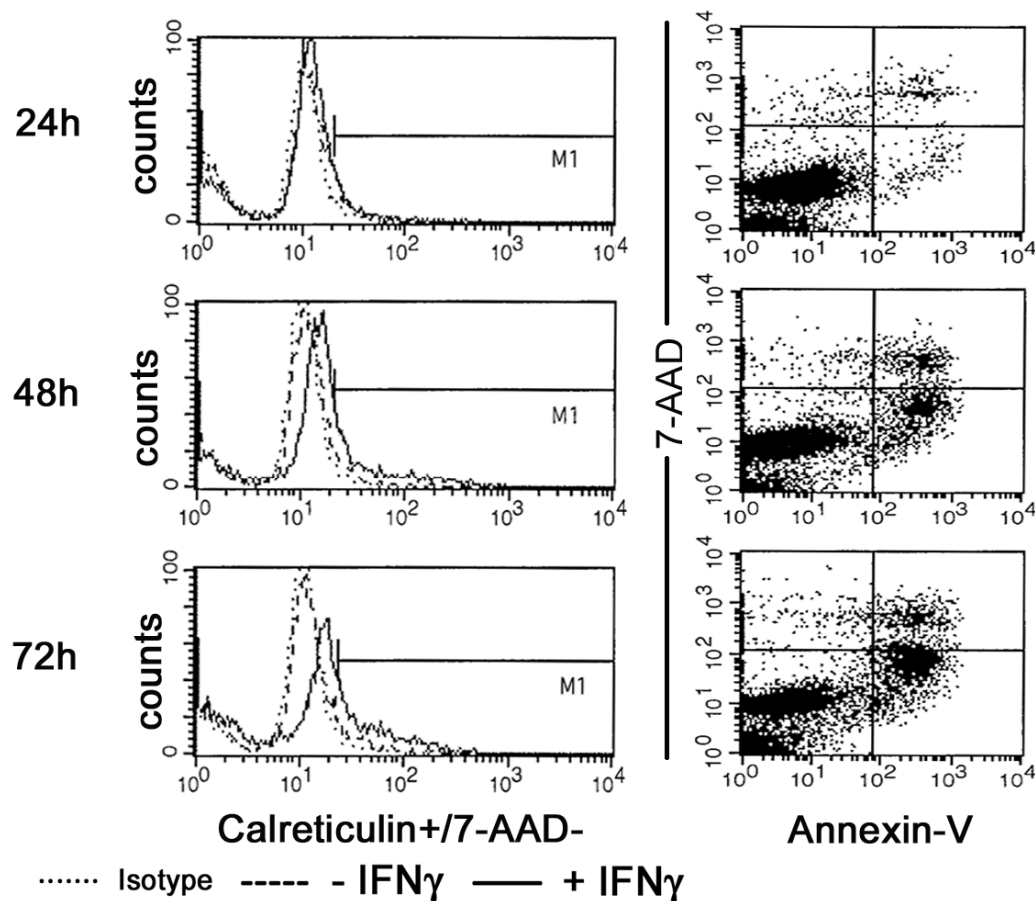
Calreticulin is not regulated by IFN- γ at the protein level



SK-MEL-28

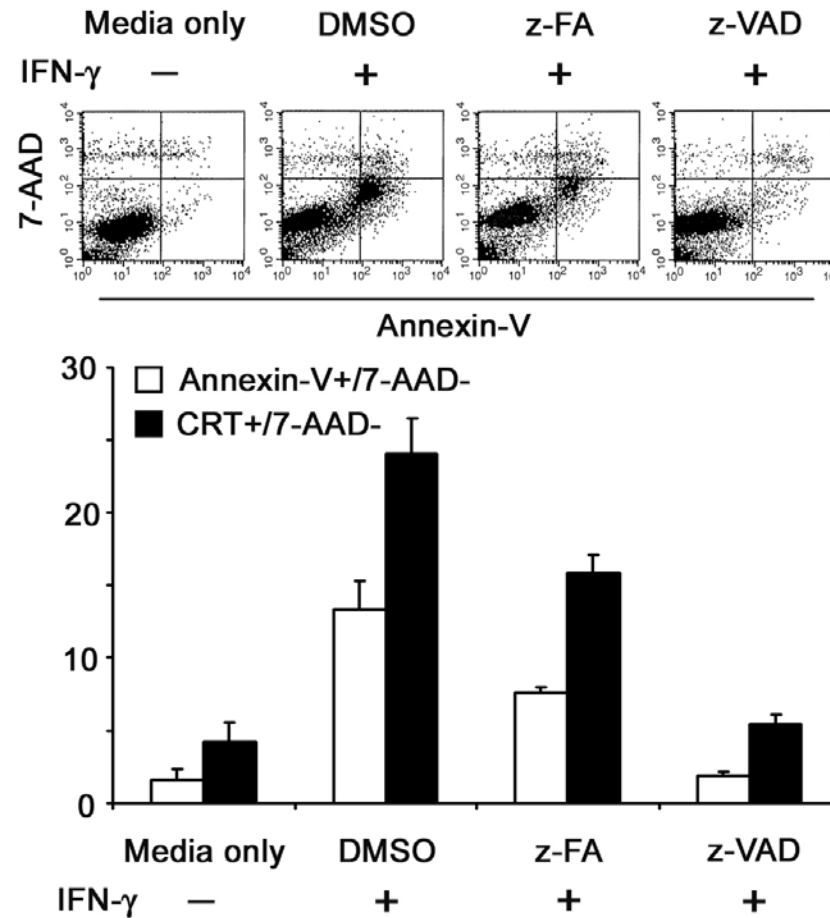
Calreticulin antibodies
IB: MBL clone FMC75
IMF: BD clone 16

Calreticulin expression and the onset of apoptosis in response to IFN- γ occur together



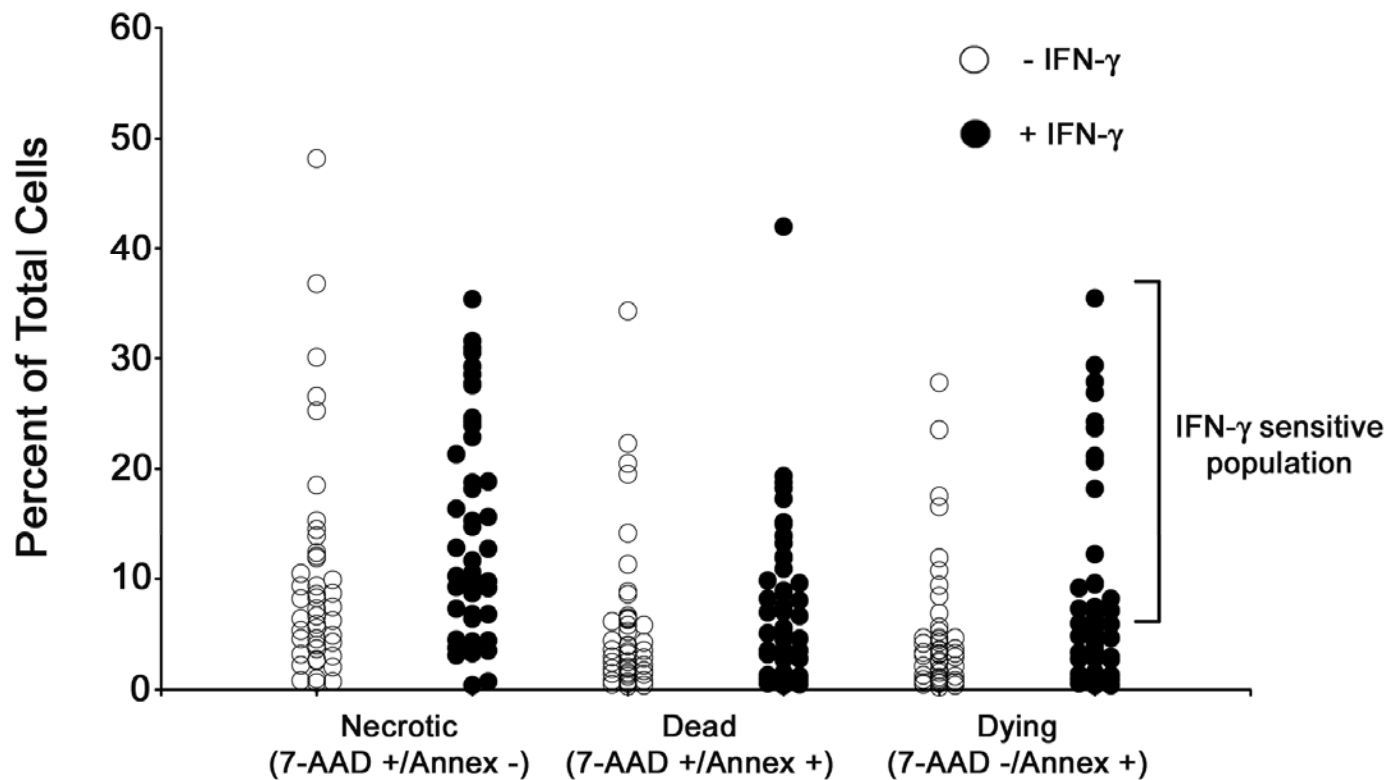
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Induction of apoptosis and calreticulin by IFN- γ is caspase dependent



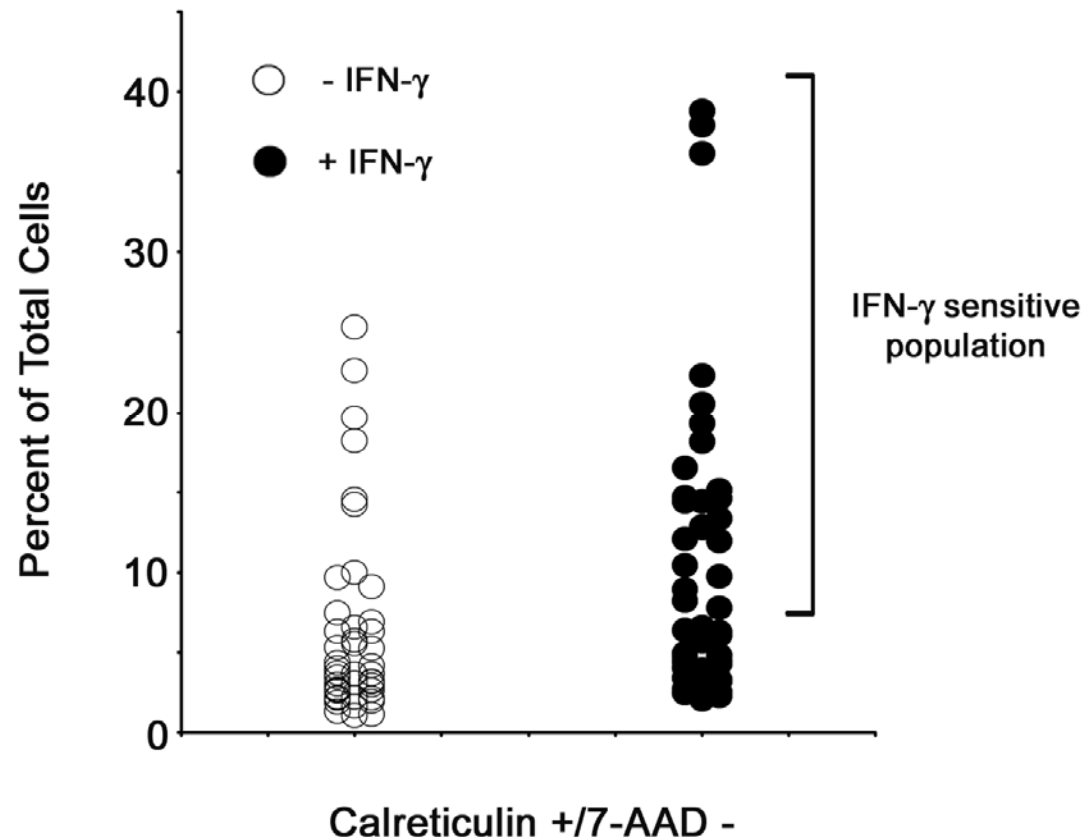
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Apoptosis is differentially induced by IFN- γ in melanoma cell lines used for loading dendritic cells



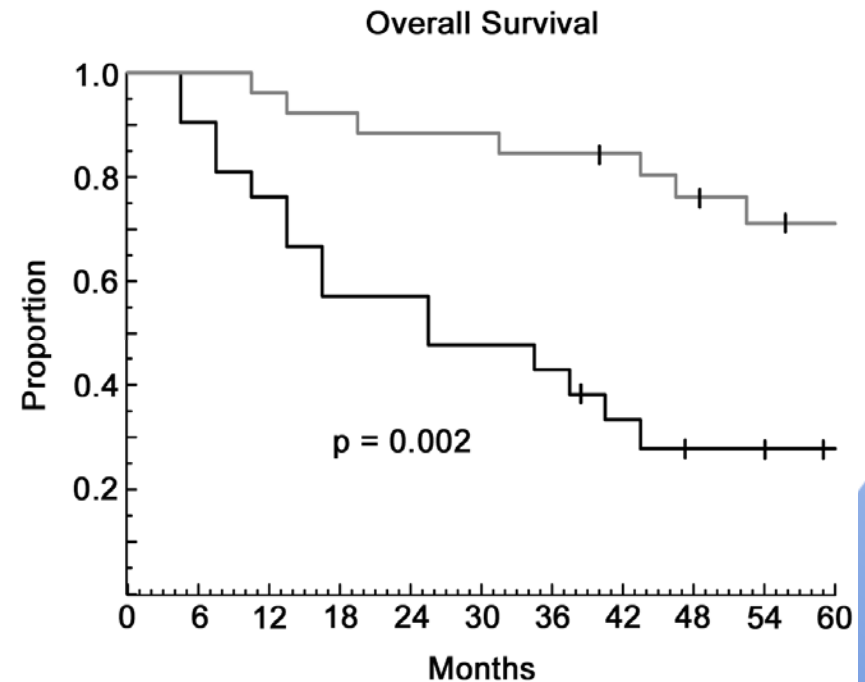
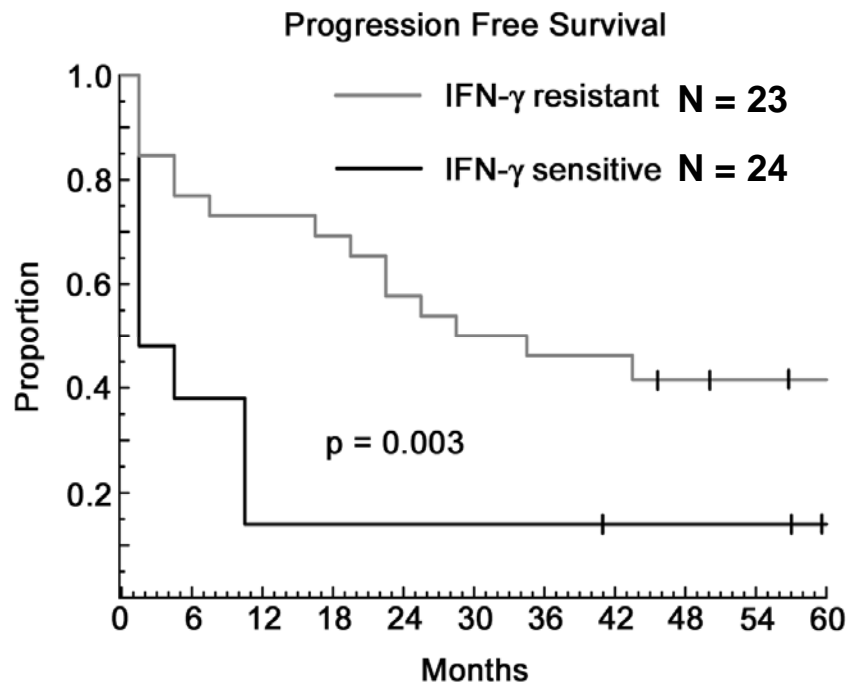
N = 47

Calreticulin exposure is increased in the IFN- γ sensitive populations



N = 47

Resistance to IFN- γ induced apoptosis is associated with survival benefit



**IFN-g resistant group: 70% are still alive,
6/10 are still disease free after >60 months**

Conclusions

- IFN- γ treatment of melanoma tumor cell lines resulted in increased amounts of phosphatidylserine and calreticulin exposure which were caspase dependent.
- Patients whose autologous cells resisted the proapoptotic effects of IFN- γ had a better outcome than those whose cells displayed apoptotic markers.
- The presence of some apoptotic cells in the vaccine preparation may be detrimental in dendritic cell therapy.

Acknowledgements

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- Principle investigators:
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- Dr. SR Selvan, PhD.
- Dr. P Schiltz, PhD.

Laboratory Staff:

- Sarah Hendry, BS.
- Denysha Carbonell, BS.
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- Maggie Hui, BS
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- Amy Mattes




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Patients divided into IFN- γ sensitive and insensitive cohorts

		All	IFN- γ Insensitive	IFN- γ Sensitive
Sex	M	29	11	18
	F	18	12	6
Age (yrs)	M	50.4 \pm 11.3	50.0 \pm 12.7	53.1 \pm 10.2
	F	46.7 \pm 16.8	51.2 \pm 16.9	44.8 \pm 17.0
	M + F	49.3 \pm 13.6	50.5 \pm 14.3	50.3 \pm 13.1
Disease Status	M1a/b	19	10	9
	M1c	28	13	15
Prior Treatment	RT	10	5	5
	Chemo	14	8	6
	IL-2	10	4	6
	IFN-alpha	17	7	10
	GM-CSF	18	8	10
	Vaccine	6	4	2
	BCG	2	0	2
	mAb	1	0	1
	Biochemo	17	10	7
DC Phenotype	%CD80	37.0 \pm 15.1	35.4 \pm 16.3	38.3 \pm 14.2
	%CD83	11.0 \pm 7.5	12.4 \pm 8.3	9.7 \pm 6.7
	%CD86	71.8 \pm 17.8	72.2 \pm 20.2	71.4 \pm 15.8
	%CD11c	88.5 \pm 8.0	88.8 \pm 7.9	88.8 \pm 9.3
IFN- γ TC	%Viability (trypan-blue)	84.9 \pm 9.3	90.0 \pm 5.5	83.0 \pm 9.9
	%CRT+/7-AAD ⁻	10.4 \pm 9.1	5.0 \pm 3.6	15.6 \pm 9.7
	%Annexin-V+/7-AAD ⁻	4.8 \pm 5.9	2.1 \pm 1.4	14.2 \pm 9.4
	%MHC I	97.8 \pm 3.1	98.8 \pm 1.3	96.8 \pm 3.9
	%MHC II	80.8 \pm 24.4	80.8 \pm 25.0	80.7 \pm 24.2
DC + TC Doses	# Received	7.5 \pm 1.2	7.7 \pm 0.9	7.2 \pm 1.4
	Cell# x 10 ⁶	16.0 \pm 7.3	15.4 \pm 6.3	16.7 \pm 8.2
	%Viability	79.7 \pm 10.3	80.0 \pm 9.8	79.5 \pm 10.8

Ave \pm SD