Immunogenic cell death governs cancer cell reprogramming and therapeutic resistance through Type-I-IFNs

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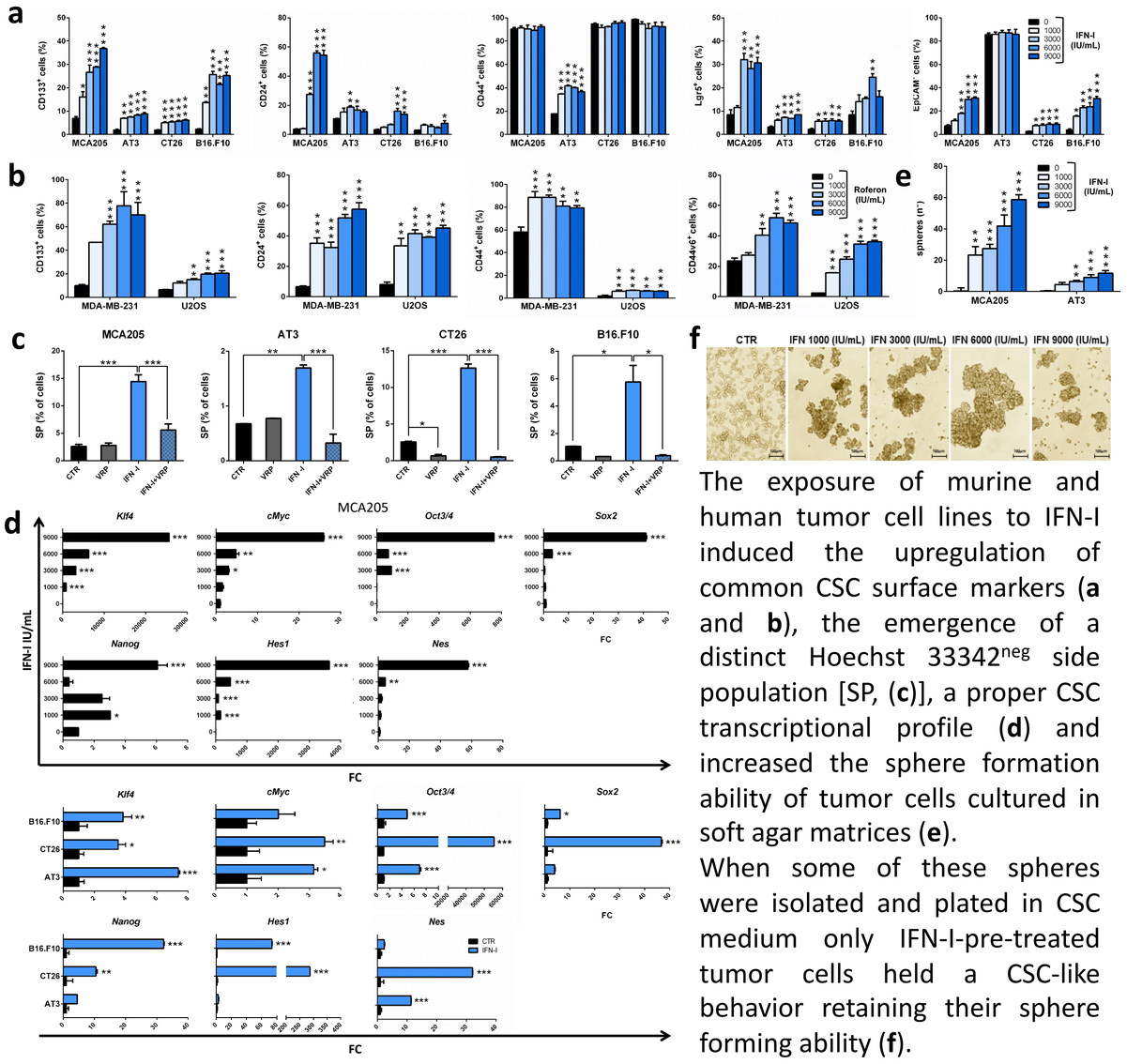
M Musella[1,2*], C Galassi[3*], G Manic[1], M Pallocca[1], A Di Benedetto[1], C Ercolani[1], P Nisticò[1], L Zitvogel[4], M Biffoni[5], R De Maria[3], I Vitale[6] and A Sistigu[1,3]

[1]IRCCS Regina Elena National Cancer Institute, Rome, Italy; [2]University of Rome "La Sapienza", Rome, Italy; [3]Catholic University "Sacro Cuore", Rome, Italy;
[4]Gustave Roussy Cancer Campus, Villejuif, France; [5]Istituto Superiore di Sanità, Rome, Italy; [6]University of Rome "Tor Vergata", Rome, Italy

The Yin-Yang of Type-I-IFNs during Immunogenic chemotherapy

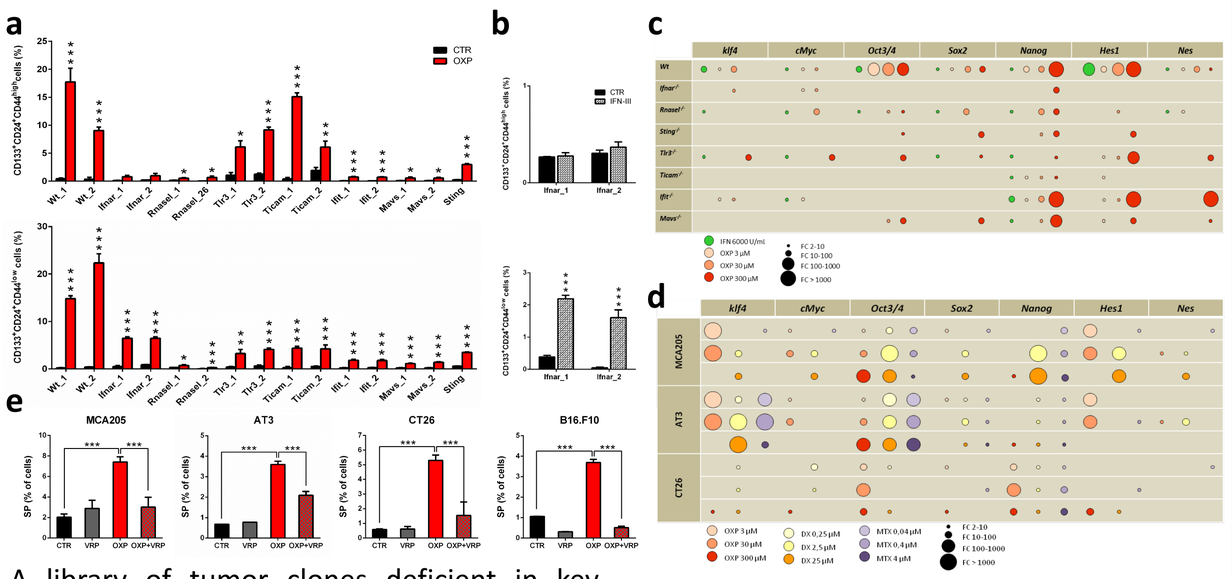
Immunogenic chemotherapy (IC) induces immunogenic cell death (ICD), which, similar to viral infection, leads to a cancer-cell autonomous Type-I-Interferon (IFN-I) signaling. Although this immunological signature is crucial for effective antitumor responses, some tumors develop resistance and relapse.
Cancer stem cells (CSCs), a niche of immature tumorigenic and immunoprivildged cells, act as propellers of metastasis and tumor relapse and are the roots of therapeutic failure. In this study, we have investigated the paradoxical role of IFN-I during IC in inducing a cancer editing program resulting in the appearance of poor immunogenic CSCs.

1. Exogenus IFN-I administration correlates with the appearance of a distinct CSC subpopulation of tumor cells



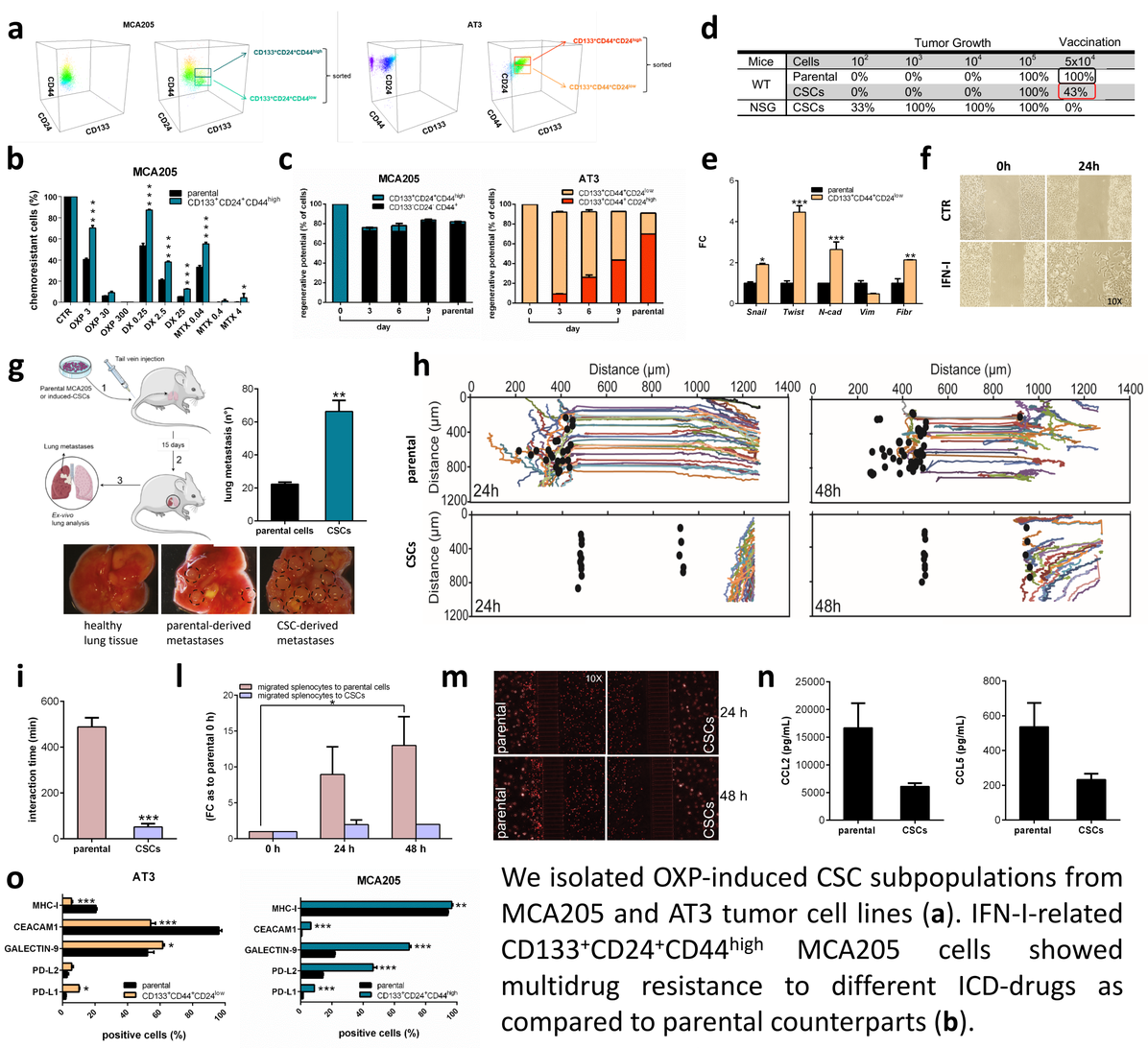
The exposure of murine and human tumor cell lines to IFN-I induced the upregulation of common CSC surface markers (**a** and **b**), the emergence of a distinct Hoechst 33342^{neg} side population [SP, (**c**)], a proper CSC transcriptional profile (**d**) and increased the sphere formation ability of tumor cells cultured in soft agar matrices (**e**).
When some of these spheres were isolated and plated in CSC medium only IFN-I-pre-treated tumor cells held a CSC-like behavior retaining their sphere forming ability (**f**).

2. IC elicits a CSC phenotype through IFN-I signaling



A library of tumor clones deficient in key elements of IFN-I net was exposed to the ICD-inducer oxaliplatin (OXP), revealing the appearance of two distinct CSC subpopulations (**a**).
Only CD133^{+}CD24^{+}CD44^{high} cells were induced in IFN-I-dependent manner as OXP and IFN-III failed to select for such as CSCs in *Ifnar*$^{-/-}$ clones (**a** and **b**, upper panel).
All clones except *Ifnar*$^{-/-}$ were endowed with a CSC transcriptional profile upon IFN-I and OXP treatment (**c**). Tumor cell lines hit with ICD-inducers [OXP, doxorubicin (DX), mitoxanthrone (MTX)] showed the same transcriptional pluripotent phenotype (**d**). OXP and DX treatment were associated with the appearance of a Hoechst 33342^{neg} SP able to efflux the drugs (**e** and **f**). Isolated MCA205 DX^{low} cells were negative for Hoechst 33342 staining and able to survive in culture as compared to DX^{high} counterparts (**g**).

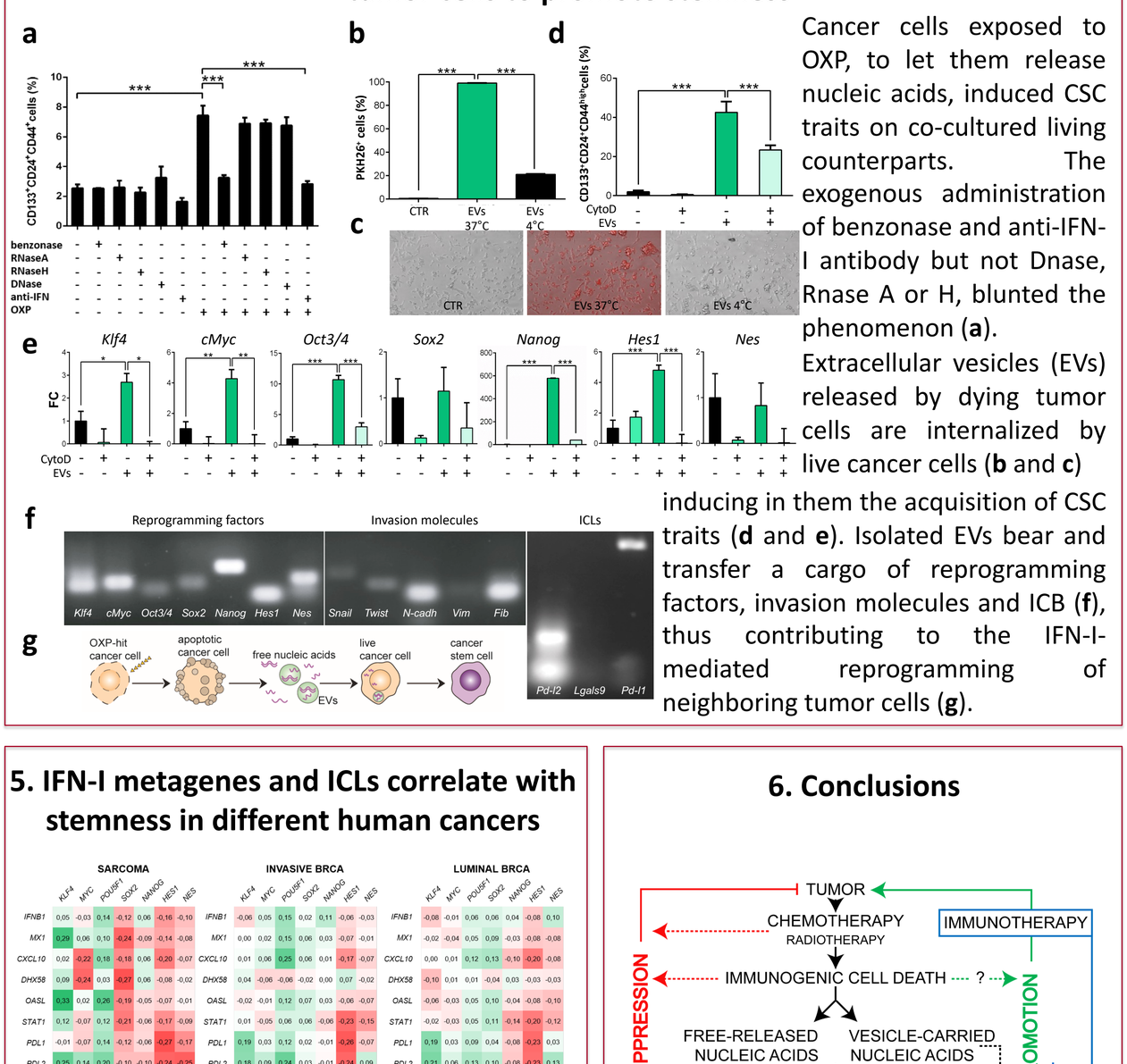
3. Functional characterization of IFN-induced CSCs



Mice	Cells	Tumor Growth 10^2	10^3	10^4	10^5	Vaccination $5x10^4$
WT	Parental	0%	0%	0%	100%	100%
	CSCs	0%	0%	0%	100%	43%
NSG	CSCs	33%	100%	100%	100%	0%

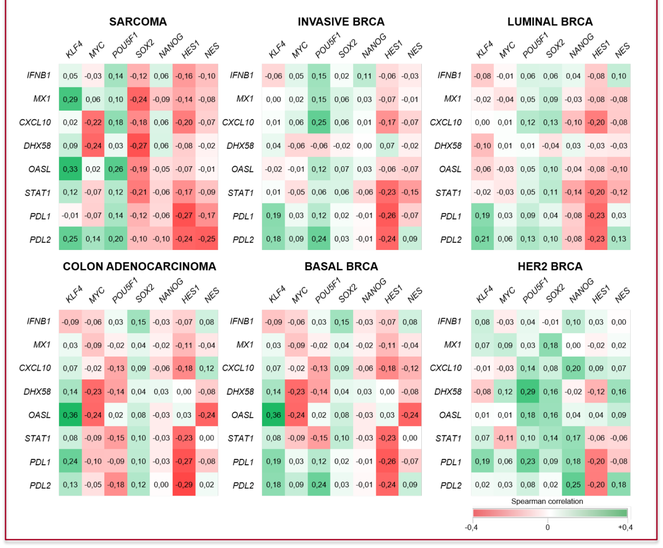
We isolated OXP-induced CSC subpopulations from MCA205 and AT3 tumor cell lines (**a**). IFN-I-related CD133^{+}CD24^{+}CD44^{high} MCA205 cells showed multidrug resistance to different ICD-drugs as compared to parental counterparts (**b**).
CD133^{+}CD24^{+}CD44^{high} MCA205 and CD133^{+}CD44^{+}CD24^{low} AT3 subsets exhibited *in vitro* tumorigenic potential (**c**). IFN-induced CSCs also displayed a strong tumorigenic capacity and a reduced vaccination potential *in vivo* (**d**) and they were dramatically invasive and metastatic as compared to parental cells (**e** - **g**).
Experiments on microfluidic devices revealed poor immunogenicity of CSCs and reduced capability to attract and interact with isolated murine splenocytes (**h-m**), further confirmed by reduced levels of CCL2 and CCL5 (**m**) and by the expression of immune checkpoint ligands [ICLs (**o**)].

4. Free and vesicle-carried nucleic acids are transferred from dying to live tumor cells to promote stemness



Cancer cells exposed to OXP, to let them release nucleic acids, induced CSC traits on co-cultured living counterparts. The exogenous administration of benzonase and anti-IFN-I antibody but not Dnase, Rnase A or H, blunted the phenomenon (**a**).
Extracellular vesicles (EVs) released by dying tumor cells are internalized by live cancer cells (**b** and **c**) inducing in them the acquisition of CSC traits (**d** and **e**). Isolated EVs bear and transfer a cargo of reprogramming factors, invasion molecules and ICB (**f**), thus contributing to the IFN-I-mediated reprogramming of neighboring tumor cells (**g**).

5. IFN-I metagenes and ICLs correlate with stemness in different human cancers



6. Conclusions