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Heavy Ion Therapy Integration with Immune and Target Drugs

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Immuno-oncology

Many cancers are not inflammed, and thus, ***not recognized by the immune system cells***.

Immune system is not blind!

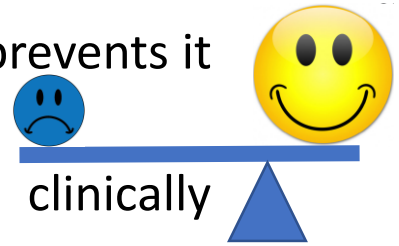
Cancer immunoediting

Cancer immunoediting: from immunosurveillance to tumor escape Gavin P. Dunn, Allen T. Bruce, Hiroaki Ikeda, Lloyd J. Old & Robert D. Schreiber

Nature Immunology **3**, 991–998 (2002)

- ***Cancer immunoediting*** is the process of interaction between the immune system and tumor cells, whereby the immune system can both **eliminate** and **promote** tumor development!
- **"3E" phases**: **elimination**, **equilibrium** and **escape**.

- The **elimination phase**: **pre-cancerous lesions eliminated** via innate and adaptive immune mechanisms.
- The **equilibrium phase** (acquisition)**:** the immune system holds the **lesion in check** and prevents it from becoming clinically detectable.
- The **escape phase:** tumor acquired potential to evade immune control becoming clinically discernible.



O'Donnell, J.S., Teng, M.W.L. & Smyth, M.J. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol 16, 151–167 (2019).

How tumors evade the host immune system

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2 strategies:

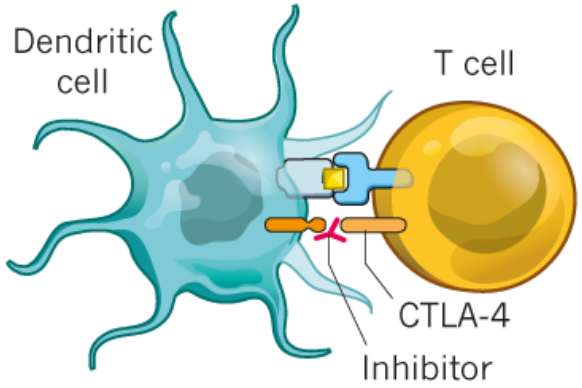
- ***avoiding recognition*** ("hiding" from immune cells) through:
- silencing or deleting the genes involved in antigen generation,
- downregulating the antigen processing and presentation on MHC-I,
- reducing MHC-I expression etc.

- ***disabling or eliminating immune cells*** through:
- up-regulation and secretion of immunosuppressive factors (IL-10, TGFb, ROS, TRAIL)
- expression of immunosuppressive molecules (PDL1)
- recruitment of immune cells (DSc, MDSCs, Tregs) that actively mediate tolerance.

**Tumors directly interfere with the host immune system, produce and release factors that modulate functions of immune cells or induce apoptosis of these cells.*

Homeostatic role of check points, Tregs, MDSC: origine of tolerance to tumor

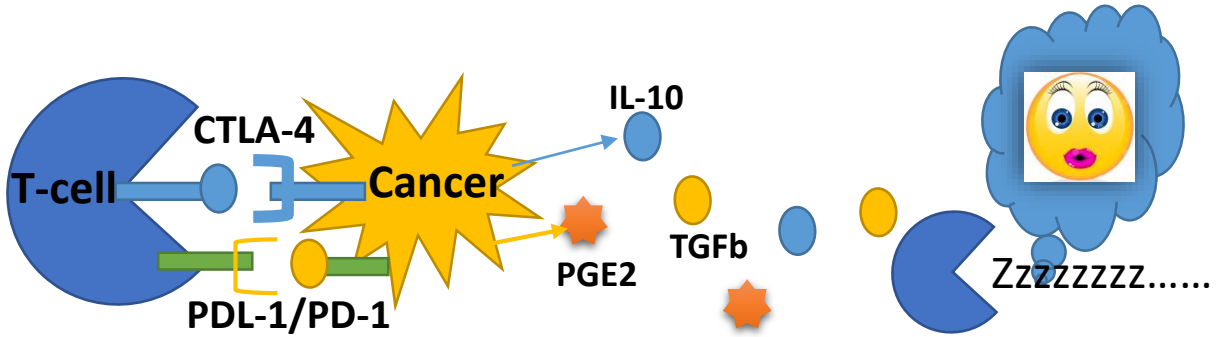
- Natural **check-point mechanisms (*CTLA-4*, *PDL/PDL-1)*** protect against unwanted immune responses.
- Operated by ***Tregs*** and ***MDSC*** **<u>normally</u>** responsible for balance in immune activity (localize normal tissue damage) following inflammation and immune recognition.



- Any immuno-stimulatory factor that generates immunity will switch on the control mechanisms including:

- *induction of PDL1/PD-1 pathways*,
- *Tregs and MDSC recruitment*,
- *release of anti-inflammatory cytokines IL-10, TGF-b aiming to counterbalance the pro-inflammatory, pro-oxidant, immune activating processes*.

Tumor-induced immunosuppression:
check points, Tregs, MDSC



Tumor cells re-educate Tregs/MDSC to establish immunosuppressive TME.

Tumor-immunosuppression includes :

-***chemokine release for recruitment of MDSC*** *and their* ***reeducation/reprogramming***,
-***production and release of immune suppressive factors:*** *TGFß, IL-10, IL-35, PGE2, ROS, TRAIL etc.*
-*Tumor-reprogrammed Tregs/MDSC-****inhibition of T cell activation***, ***function*** and ***trafficking*** (adaptive immunity),
-*Tumor-induced MDSC-****inhibition of innate anti-tumor immunity*** by ***polarizing macrophages*** towards M2,

- *Reprogrammed MDSC facilitate tumor growth by supporting neoangiogenesis, tumor cell invasion and metastasis through production of matrix metalloproteinase-9, VEGF.*

Example *of* ***functional aberrations of TILs*** *freshly isolated from human tumors (1): compared to those distant from tumor site, these TILs from tumor site are unresponsive to traditional T-cell activating stimuli, producing more immunosuppressive cytokines!*

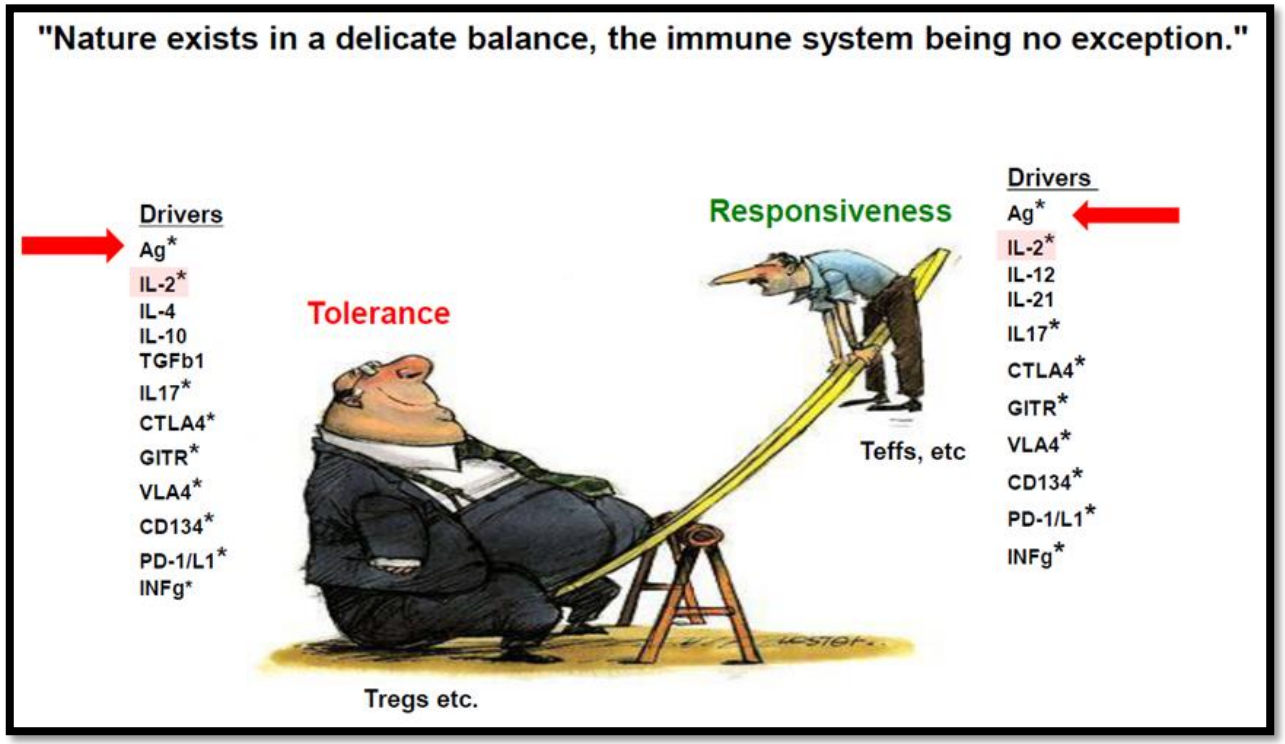
Whiteside TL. Tumor infiltrating lymphocytes in human malignancies. Austin, TX: RG Landes Co.; 1993.

Immune paradox

Bimodality of ***cytokines*** and ***immune cells:*** immunosuppressive and immunostimulatory functions.

IMMUNE CELLs: ***Macrophages*** (M1/2), ***Lymphocytes*** (Th1/2), ***MDSCs***,

CYTOKINES: IFN I, IFN II TGFb1 IL-2 IL-10



Lee AJ, Ashkar AA. The Dual Nature of Type I and Type II Interferons. Front Immunol. 2018 Sep 11;9:2061. doi: 10.3389/fimmu.2018.02061. PMID: 30254639; PMCID: PMC6141705.

Han G, Li F, Singh TP, Wolf P, Wang XJ. The pro-inflammatory role of TGFβ1: a paradox? Int J Biol Sci. 2012;8(2):228-35. doi: 10.7150/ijbs.8.228. Epub 2012 Jan 1. PMID: 22253566; PMCID: PMC3258562.

Fioravanti J, Di Lucia P, Magini D, et al. Effector CD8+ T cell-derived interleukin-10 enhances acute liver immunopathology. J Hepatol. 2017 Sep;67(3):543-548. doi: 10.1016/j.jhep.2017.04.020. Epub 2017 May 5. PMID: 28483675; PMCID: PMC7127652.

Tumor Microenvironment (TMN):
network of opposing forces

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Radiation & Inflammation

Semin Radiat Oncol. 2015 January ; 25(1): 4–10. doi:10.1016/j.semradonc.2014.07.007.

Dörthe Schaue[1], **Ewa D. Micewicz**[1], **Josephine A. Ratikan**[1], **Michael W. Xie**[1], **Genhong Cheng**[2], and **William H. McBride**[1,*]

[1]Department of Radiation Oncology, University of California at Los Angeles, CA, USA

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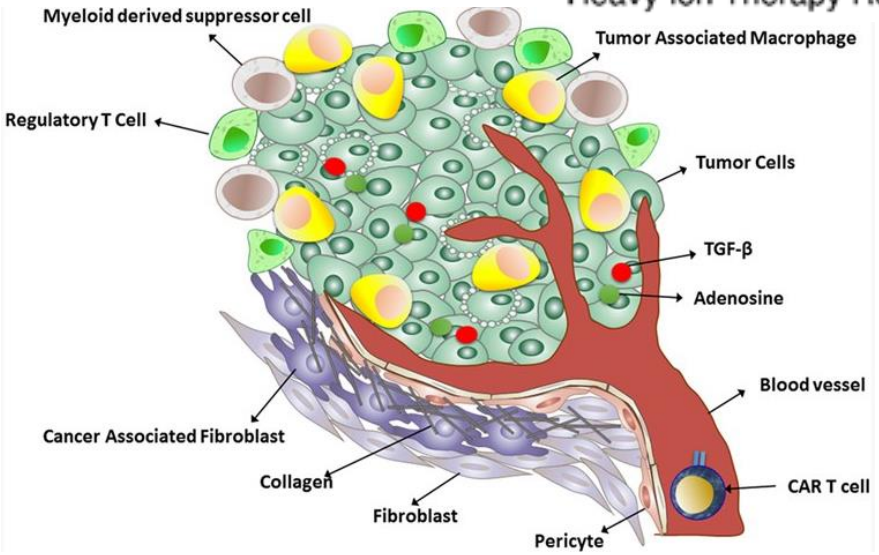

<u>MDSCs-key regulators of the TME:</u>

- attracted from bone marrow by chemokines to homing in solid tumors,
- differentiate into macrophages and granulocytes,
- exert inhibitory effects: expression of immunosuppressive molecules resulting in a **<u>decreased Teff</u>** and **<u>increased number of Tregs</u>**.

<u>TAMs</u> 2 phenotypes depending on signals from TUMOR/TME:

- **M1 "killer" type/pro-inflammatory**: releasing ROS/IL-12 that enhances antigen-specific responses, induced by Toll-like receptors and IFNg,
- **M2 "healer" type/immunosuppressive:** HIF-1,-ß arginase, IL-10, IL-6 and VEGF push angiogenesis, wound healing, tumor invasion.

*Example: cancer cell-derived-**G protein-coupled receptor 132 ligand (Gprl132)** can educate macrophages to become functional M2-type.*

Tartour, E., Pere, H., Maillere, B. et al. 2011. Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. Cancer Metastasis Rev. 30:83.

Chen, P. and Bonaldo, P. 2013. Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. Int. Rev. Cell Mol. Biol. 301:1.

Colegio, O. R., Chu, N. Q., Szabo, A. L. et al. 2014. Functional polarization of tumour-associated macrophages by tumour derived lactic acid. Nature 513:559.

Chen, J., Yao, Y., Gong, C. et al. 2011. CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3. Cancer Cell 19:541.

The suppressive power of TMN

[CANCER RESEARCH 50, 2228–2233, April 15, 1990]

Radiosensitive Barrier to T-Cell-mediated Adoptive Immunotherapy of Established Tumors[1]

Michel Awwad and Robert J. North[2]

Trudeau Institute, Saranac Lake, New York 12983

1.) **Adoptive Immunotherapy** is **possible in irradiated**, **but not in immunocompetent recipients**:

- It is not possible to cause regression of the **immunogenic SA-1 sarcoma** by adoptive immunotherapy with **tumor sensitized T-cells**, unless the **tumor-bearing recipient is exposed to a sublethal dose of irradiation to remove a barrier** that prevents adoptive immunity from being expressed.

2.) **Regeneration of the Barrier in Irradiated Mice Is Tumor-induced and Thymus Dependent**:

- *This barrier to adoptive immunotherapy was found to be regenerated between 2 and 4 weeks following irradiation, and its regeneration was associated with general repopulation of host T cells. However, it was not regenerated in the absence of the thymus, thus showing that it is T-cell dependent.*

3.) **The Barrier to Adoptive Immunotherapy Coexists with Host Concomitant Immunity**:

- *Evidence that it is caused by the presence of CD4 suppressor T-cells was shown by the finding that it can be removed by depleting mice of **CD4 T-cells** with **monoclonal antibodies**, but not by depleting them of CD8 T-cells. Again, the barrier could be restored to irradiated recipients by infusing them with **CD4 T-cells**, but not with CD8 T-cells, from tumor-bearing donors.*

4.) **Depletion of CD4 + T-Cells Removes the Barrier to Adoptive Immunotherapy**:

- ***Cyclophosphamide-facilitated adoptive immunotherapy*** of a ***cyclophosphamide-resistant tumor***. Evidence that cyclophosphamide permits the expression of adoptive T-cell-mediated immunity by removing suppressor T-cells rather than by reducing tumor burden.

Tumor temperature: hot or cold?

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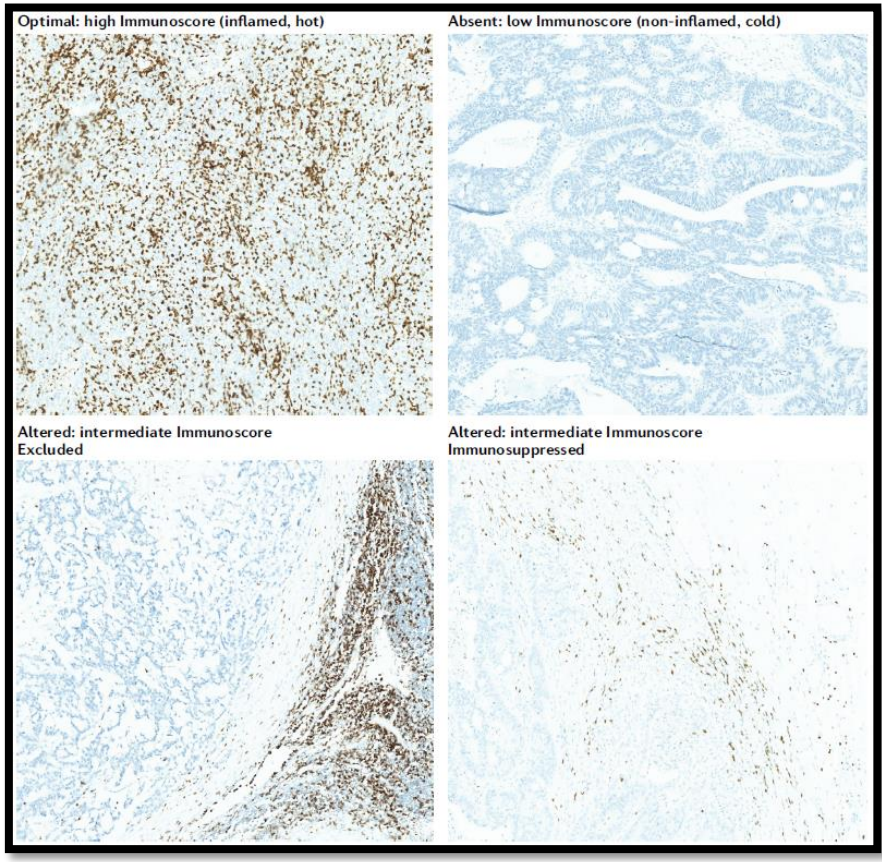
Immunophenotypes: immune-inflamed/HOT, and immune-desert/COLD.

IMMUNO-PHENOTYPE	INFLAMMATION	INFILTRATING T-cells	MUTATIONAL BURDEN	NEOANTIGENS	PD-L1 expression	RESPONSE to ICIs	TME-cells
HOT	yes	yes	high	yes	yes	yes	Th1, M1, CD8+
COLD	no	no	low	no	no	no	MDSC, Tregs, M2, Th2



- **<u>"HOT" tumors:</u>**
  melanoma, bladder, kidney, H&N, NSCLC, liver.

- **<u>"COLD" tumors:</u>**
  breast cancers, ovarian cancer, prostate cancer, pancreatic cancer, glioblastomas, colorectal.

- At the time of cancer diagnosis cold tumors constitute the most frequent phenotype.

Reasons for being cold

Absence of the T-cells in tumor can be due to:

- **Lack of tumor antigens**: silencing/deleting the genes, downregulating the antigen processing and reduced MHC-I expression.
- **Defect in antigen presenting cells**: suppression by Tregs, MDSCs, DCs.
- **Absence of T cell activation**: defective recruitment and activation of APCs.
- **Absence of T cell infiltration and deficit of homing into the tumor bed**: immunosuppressive peritumoral stroma, tumor cell and vessels alteration, b-catenin and PI3K pathway activation/PTEN loss, TGFb, CXCL-9/10, CXCL16 etc.

Courtesy of Prof. Depil

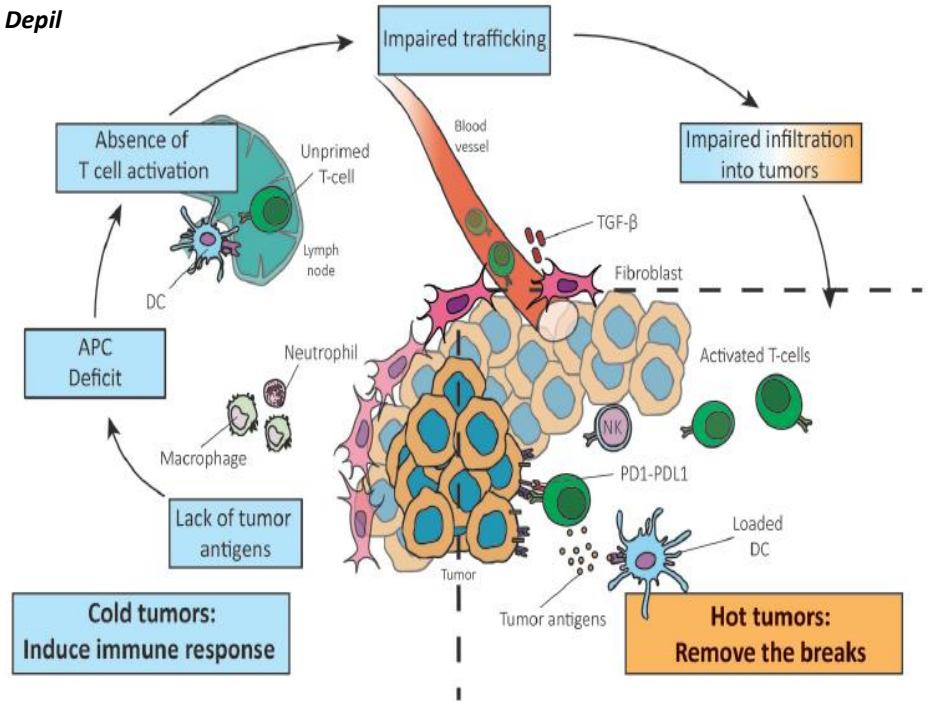


FIGURE 1 | Reversing a cold into a hot tumor. Adapted from Chen and Mellman (1). The absence of T cells in the tumor can be due to the lack of tumor antigens, APC deficit, absence of T cell priming/activation and impaired trafficking of T cells to the tumor mass (left panel). Understanding which step of the anti-cancer immune response is not functional in cancers is crucial to adapt therapies to the cancer phenotype.

Tumor temperature:

significance of TILs and TDL Immune classification

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- **TDL Immune Classification: Type**, **Density** and **Location** of immune cells within the tumor site.
- Can predict survival, local control or response to CHT/IT independently and more accurately than TNM system!
- Validated for CRC, melanoma.
- **Immunoscore**: scoring system based on the *quantification of two lymphocyte populations* (**CD3** and **CD8**) at the *tumor center* and the *invasive margin*.

The Immunoscore ranges 0-4:

- **0** (absence of both cell types in both regions), immuno-desert cold tumors,
- **2** (single cell type in single region), immuno-excluded phenotype,
- **4** (high immune cell densities in both locations), inflamed, hot tumors.

Example: in CRC the 2-year risk of relapse for these three types of tumor was 80%, 50% and 10%, respectively.

1. El Sissy C, Kirilovsky A, Zeitoun G, Marliot F, Haicheur N, Lagorce-Pagès C, Galon J, Pagès F. Therapeutic Implications of the Immunoscore in Patients with Colorectal Cancer. Cancers. 2021; 13(6):1281. https://doi.org/10.3390/cancers13061281

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Immunogenicity of Radiation Therapy

Rational for combining RT and IT:
Stimulatory immunogenic effects of conventional RT

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Immunostimulatory Effects	
Calreticulin translocation to the surface of tumor cells ("eat me" signal) *	Increased tumor cells phagocytosis Promotes pro-inflammatory cytokines release from APCs
Release of HMGB1 protein ("danger signal") *	DC migration and maturation (increase in efficiency of antigen processing and presentation) Release of pro-inflammatory cytokines and chemokines from APCs
Release of ATP *	Release of pro-inflammatory cytokines from APCs (priming of IFN-γ-producing cytotoxic CD8+ T cells)
HSP increase (membrane-bound expression and extracellular release) *	Stimulate innate and adaptive immune responses
Decrease of CD47 surface expression ("do not-eat-me" signal)	Increase tumor cells phagocytosis
Accumulation of cytosolic DNA in irradiated tumor cells *	Activation of the cGAS/STING pathway and production of type I IFNs and other pro-inflammatory cytokines (APCs maturation, cross-presentation and T cell recruitment)
Smac release from mitochondria	Increase tumor cells sensitivity to granzyme-induced apoptosis
Generation of novel peptides and increase of the pool of intracellular peptides presented	Increase the anti-tumor immune response
Increased MHC-I expression (critical for antigen recognition by CD8+ TCRs)	Enhance recognition and killing of cancer cells by cytotoxic T cells
Increase of NKG2D ligands, co-stimulatory molecules (e.g., CD80) and adhesion molecules (e.g., ICAM-1, E-selectin) on tumor cells	Enhance recognition and killing of cancer cells by cytotoxic lymphocytes
Upregulation of "death receptors" (e.g., FAS/CD95)	Enhance recognition and killing of cancer cells by cytotoxic lymphocytes
Release of chemokines (e.g., CXCL9, CXCL10, CXCL16,), increase of adhesion molecules on the vascular endothelium (e.g., VCAM-1), normalization of the tumor vasculature	Facilitate the recruitment of effector T-cells to the tumor site

Demaria S, et al. Immune-mediated inhibition of metastases following treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res. 2005;11:728–734

Dewan MZ, et al. Fractionated but not single dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res. 2009;15:5379–5388.

Golden EB, et al. An Abscopal Response to Radiation and Ipilimumab in a Patient with Metastatic Non-Small Cell Lung Cancer. Cancer Immunol Res. 2013;1:365–372.

Germano, G., Lamba, S., Rospo, G., Barault, L.,Magrì, A.,Maione, F., et al. (2017). Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. Nature 552, 116–120. doi: 10.1038/nature24673

Rational for combining RT and IT ??

Suppressive immunogenic effects of conventional RT

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Immunosuppressive Effects	
Upregulation of PDL-1 on cancer cells	Inhibit CTL-mediated tumor killing
Accumulation of regulatory T cells (related to intrinsic higher radio-resistance and increase of immunosuppressive mediators and cytokines induced by radiation)	Immunosuppression
Accumulation of immunosuppressive myeloid cells (N2 neutrophils, M2 macrophages, MDSCs) secondary to the increase of CSF-1, SDF-1, CCL2 induced by radiation	Immunosuppression
Induction of TGF-beta secretion	Multiple immunosuppressive effects
Upregulation of the transcription of HIF-1α	Multiple immunosuppressive effects
Upregulation of adenosine	Multiple immunosuppressive effects
Killing of tumor-infiltrating immune cells (e.g., lymphocytes, APCs)	Immunosuppression

Demaria S, et al. Immune-mediated inhibition of metastases following treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res. 2005;11:728–734

Dewan MZ, et al. Fractionated but not single dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res. 2009;15:5379–5388.

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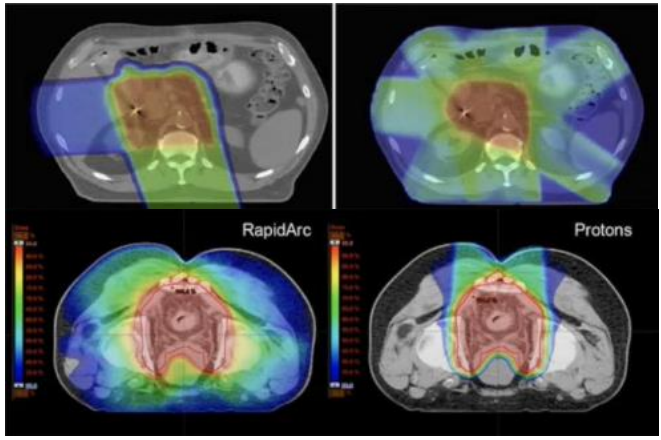
Rational for using particle therapy with IT

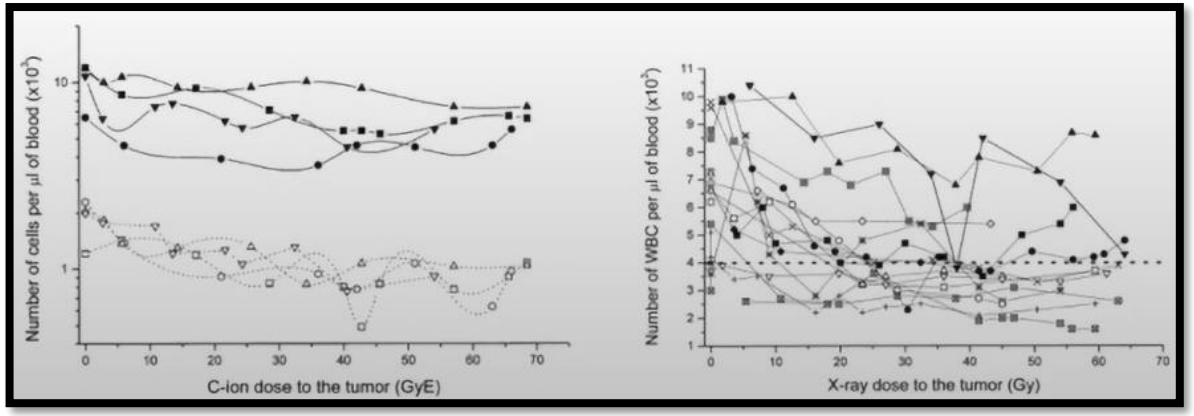
Physics of particles:

- Sparing of the immune cells (circ. Lymphocytes, loco-regional APC. TAM, TIL)

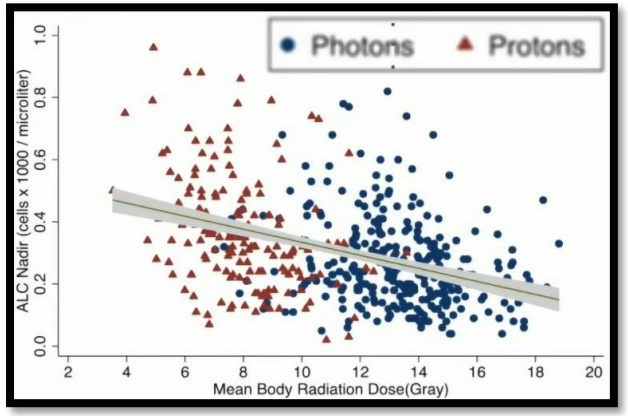
Biology of particles:

- Different cell death patterns (apoptosis, necroptosis, ceramide path)
- Increased antigenicity (high-LET-induced mutations)
- Increased adjuvanticity (higher HGMB1)





Durante et al. 2000

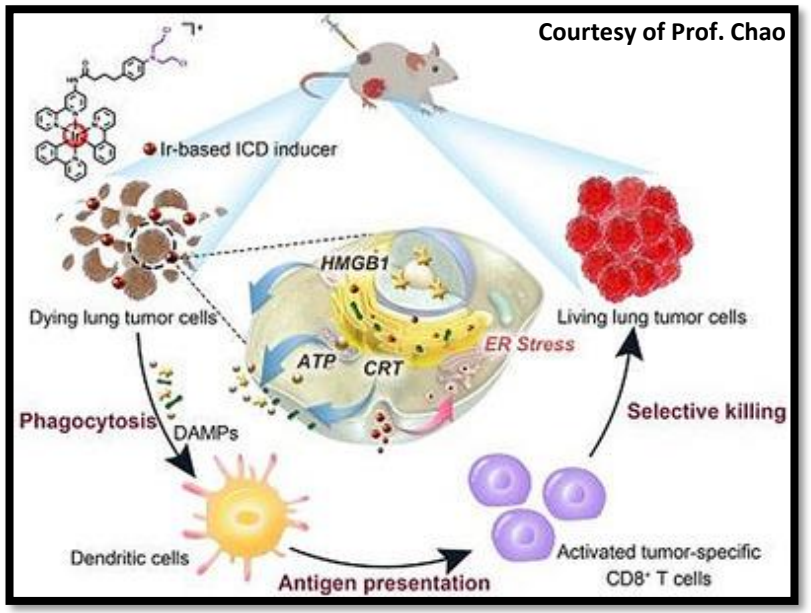


Davuluri et al. 2017

Immunogenic effects of RT:

Immunogenic cell death (ICD)

- Tumor cell death resulting in an immune response.
- ICD comprises the release of **DAMPs: ATP, HMGB1, calreticulin and heat shock proteins** resulting in the activation of tumor-specific immune responses.
- Radiation-induced DNA damage (nuclear fragmentation, micronuclei) activates **the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) *pathway***, resulting in **INTERFERON-1** production leading to upregulation of DAMPs, recruitment of APCs and subsequent adaptive immune response.
- **STING pathway plays a central role in anti-tumor immunity** and its expression is lost in several cancer types.



Both photon and particle radiation have been reported as bona fide inducers of ICD

Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G. M., Apetoh, L., Perfettini, J. L., et al. (2007). Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat. Med. 13, 54–61. doi: 10.1038/nm1523
Golden, E. B., Pellicciotta, I., Demaria, S., Barcellos-Hoff, M. H., and Formenti, S. C. (2012). The convergence of radiation and immunogenic cell death signaling pathways. Front. Oncol. 2:88. doi: 10.3389/fonc.2012.00088
Yoshimoto, Y., Oike, T., Okonogi, N., Suzuki, Y., Ando, K., Sato, H., et al. (2015). Carbon-ion beams induce production of an immune mediator protein, high mobility group box 1, at levels comparable with X-ray irradiation. J. Radiat. Res. 56, 509–514. doi: 10.1093/jrr/rrv007
Demaria, S., Ng, B., Devitt, M. L. et al. (2004). Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int. J. Radiat. Oncol. Biol. Phys. 58, 862–870. doi: 10.1016/j.ijrobp.2003.09.012
Deng, L., Liang, H., Xu, M., Yang, X., Burnette, B., Arina, A., et al. (2014b). STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 41, 843–852. doi: 10.1016/j.immuni.2014.10.019

IMMUNOGENICITY OF CARBON-Ion THERAPY: experimental studies

Comparison of the effects of photon, proton and carbon-ion radiation on the ecto-calreticulin exposure in various tumor cell lines

Yangle Huang[1,2], Yuanli Dong[1,2], Jingfang Zhao[2,3], Lijia Zhang[2,3], Lin Kong[1,2], Jiade Jay Lu[1,2]

Ann Transl Med. 2019 Oct; 7(20): 542. doi: 10.21037/atm.2019.09.128

"Proton and photon irradiation showed a similar effectiveness increasing calreticulin exposure with dose escalation inducing the highest value at 10 Gy, while carbon-ion increased most calreticulin exposure at 4 Gy having significantly stronger immunogenic effects than proton and photon."

Source, y	Cell line	Mouse strain	Radiation therapy	Immunotherapy	Immunotherapy administration	Effect
Matsunaga et al, 2010 [47]	SCC VII; FM3A, mammary carcinoma	C3H/He; BALB/c–nude	290 MeV/n C-ion; 77 keV/μm; <10 Gy/min	BM-derived; DCs (SCC VII lysate treated, then rSeV/dF infected)	Immunotherapy, d 2, 9, 17 after IR	Second tumor rejection
Ohkubo et al, 2010 [48]	NR-S1, SCC	C3H/He	290 MeV/n C-ion; 6 cm SOBP; 6 Gy	BM-derived; α-GalCer–pulsed DCs	Immunotherapy, d 1.5 after IR	Lung metastasis

Basic research on CIRT combined with IT

IMMUNOGENICITY OF CARBON-Ion THERAPY: experimental studies

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Carbon-Ion Beam Treatment Induces Systemic Antitumor Immunity Against Murine Squamous Cell Carcinoma

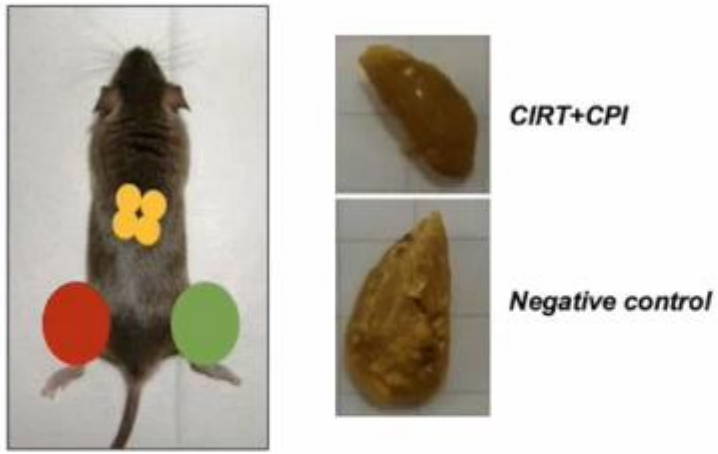
Akinao Matsunaga, MD, PhD[1,2]; Yasuji Ueda, PhD[1]; Shigeru Yamada, MD, PhD[3]; Yui Harada, PhD[1]; Hideaki Shimada, MD, PhD[2]; Mamoru Hasegawa, PhD[4]; Hirohiko Tsujii, MD, PhD[3]; Takenori Ochiai, MD, PhD[2]; and Yoshikazu Yonemitsu, MD, PhD[1,5]

Combining Carbon Ion Radiotherapy and Local Injection of α-Galactosylceramide–Pulsed Dendritic Cells Inhibits Lung Metastases in an *In Vivo* Murine Model

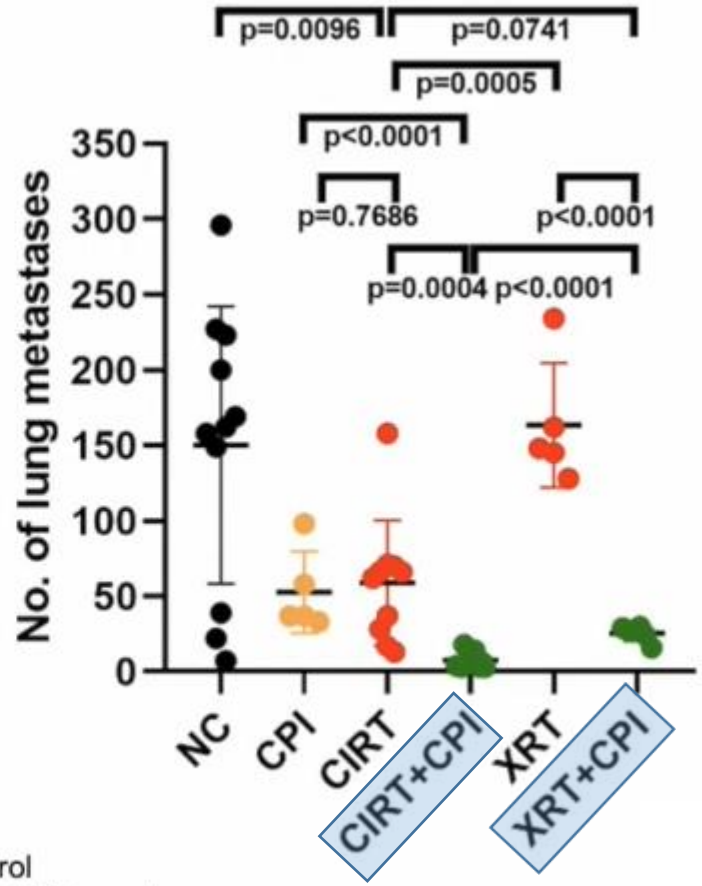
Yu Ohkubo, M.D. • Mayumi Iwakawa, M.D., Ph.D. • Ken-Ichiro Seino, M.D., Ph.D. • ...
Etsuko Nakamura, M.S. • Takashi Nakano, M.D., Ph.D. • Takashi Imai, Ph.D. • Show all authors

- CIRT + dendritic cell injection=stronger immune activation!
- Combined CIRT-IT = increased antitumor immunity and reduced the number of metastases compared with RT or IT alone, or in combination with photons.

IMMUNOGENICITY OF CARBON-Ion THERAPY: experimental studies



Assessment of superficial nodules after fixation in Bouin's solution



Helm et al., *Int. J. Radiat. Oncol. Biol. Phys.* 2021

NC = negative control
CPI = check point inhibitors only
CIRT = C-ions only, 10 Gy
XRT = photons, 10 Gy

COMBINED RT-IT: AVAILABLE CLINICAL TRIALS

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- Clinical trials using photon RT+IT resulting in increased patients' survival:

Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC

Scott J. Antonia, M.D., Ph.D., Augusto Villegas, M.D., Davey Daniel, M.D., David Vicente, M.D., Shuji Murakami, M.D., Rina Hui, Ph.D., Takayasu Kurata, M.D., Ph.D., Alberto Chiappori, M.D., Ki H. Lee, M.D., Ph.D., Maike de Wit, M.D., Ph.D., Byoung C. Cho, M.D., Ph.D., Maryam Bourhaba, M.D., et al., for the PACIFIC Investigators*

Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials

Willemijn S M E Theelen, MD * • Dawei Chen, MD * • Vivek Verma, MD • Brian P Hobbs, PhD • Heike M U Peulen, MD • Prof Joachim G J V Aerts, MD • et al. Show all authors • Show footnotes

Examples of negative trials:

Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial

Prof Nancy Y Lee, MD • Prof Robert L Ferris, MD • Amanda Psyrri, MD • Prof Robert I Haddad, MD • Makoto Tahara, MD • Prof Jean Bourhis, MD • et al. Show all authors • Show footnotes

Durvalumab plus tremelimumab alone or in combination with low-dose or hypofractionated radiotherapy in metastatic non-small-cell lung cancer refractory to previous PD(L)-1 therapy: an open-label, multicentre, randomised, phase 2 trial

Jonathan D Schoenfeld, MD • Anita Giobbie-Hurder, MS • Srinika Ranasinghe, PhD • Katrina Z Kao, BA • Ana Lako, PhD • Junko Tsuji, PhD • et al. Show all authors • Show footnotes

Radiation form? Radiation Dose? Timing? Sequencing?

Dose

the ideal immunogenic radiation dose has not been established!

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	First autor	Tumor (cells)	Dose fractionation	Immunogenic effects
LOW, sub-conventional	Tubin, S et al.	prostate (DU-145)	**0.5Gy x 1** vs. 1-30Gy x 1	Bystander-mediated tumor cell killing
	Aryankalayil,M et al.	prostate	**1Gy x 10** vs. 10Gy x 1	more robust release of DAMPs
MODERATE, hypofractionated	Dewan, MZ et al.	breast	**6Gy x 5**, **8Gy x 3** vs. 20Gy x 1	systemic anti-tumor immune responses
	Morisada, M et al.	oral	**8Gy x 2** vs. 2Gy x 10	CD8+ T cell infiltration and reduced MDSC accumulation
ABLATIVE, extremely hypofractionated	Garcia-Barros, M et al.	endothelium	**>8-10Gy x 1**	destruction of the tumor vasculature-endothelial apoptosis
	Camphausen, K et al.	lung	**10Gy x 5** vs. 2Gy x 12	robust abscopal effects
	Lugade, A. A et al.	melanoma (B16-OVA)	**15Gy x 1** vs. 5Gy x 3	higher secretion of interferon-g with more tumor-infiltrating T cells
	Tubin, S et al.	prostate (PC-3)	**20Gy x 1** vs. 0.5-30Gy x 1	Bystander-mediated tumor cell killing
	Lee, Y/Verbrugge, I	breast, lung, melanom	**>20Gy**	increase T cell priming, CD8+ T cell infiltration
	Filatenkov, A et al.	colon	**30Gy x 1** vs. 3Gy x 10	increase in CD8+ infiltration, MDSC depletion

Tsai MH, Cook JA, Chandramouli GV, DeGraff W, Yan H, Zhao S, et al. Gene expression profiling of breast, prostate, and glioma cells following single versus fractionated doses of radiation. Cancer Res. 2007;67:3845–52.

Dose-dependent radiation-immunogenic effects

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- **Reprogramming TAMs** and **vascular normalization**: **low dose radiation 0.5≤2 Gy**.
- **Upregulation of MHC-I on tumor cells: >4 Gy x 1** (*melanoma*), **>8–20 Gy x 1** (*colon cancer*).
- **Destruction of the tumor vasculature-endothelial apoptosis: ablative doses > 8-10 Gy**.
- **Radiation-damaged DNA processing: cGAS-STING** vs. **Trex1*: 20-30 Gy x 1**.

* *DNA exonuclease-Trex1-mediated degradation of cytosolic DNA consequently abrogates cGAS-STING activation and downstream IFN-1 production.*

Example: CTLA-4 blockade did not synergize with high dose irradiation to induce abscopal effects. However, knockdown of Trex1 reinstated synergistic effects of anti-CTLA-4 in combination with high dose radiation (20Gy) .

M. Garcia-Barros, F. Paris, C. Cordon-Cardo, D. Lyden, S. Rafii, A. Haimovitz- Friedman, et al., Tumor response to radiotherapy regulated by endothelial cell apoptosis, Science 300 (5622) (2003) 1155–1159.

M. Morisada, P.E. Clavijo, E. Moore, L. Sun, M. Chamberlin, C. Van Waes, et al., PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation, OncoImmunology 7 (3) (2018), e1395996.

E.A. Reits, J.W. Hodge, C.A. Herberts, T.A. Groothuis, M. Chakraborty, E. K. Wansley, et al., Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy, J. Exp. Med. 203 (5) (2006) 1259–1271.

Timing of RT delievering

- *If given following antigen delivery/release, **RT can mature DCs** and enhance their ability to cross-present immunodominant MHC-Class I peptides so as to generate superior immunity and tumor rejection.*
- *However, irradiation of DCs prior to delivery of whole antigen by adenoviral vectors, blocks their ability to generate immunity.*

The same effects can exert the cytokines depending on the timing, how the target cellular components have been programmed by the tumor or Tregs etc.

Immune Checkpoint Blockade to Improve Tumor Infiltrating Lymphocytes for Adoptive Cell Therapy. Krithika N. Kodumudi, Jessica Siegel, Amy M. Weber, Ellen Scott, Amod A. Sarnaik, Shari Pilon-Thomas
McBride WH, Howie SE. Induction of tolerance to a murine fibrosarcoma in two zones of dosage—the involvement of suppressor cells. Br J Cancer 1986; 53:707–11. doi: https://doi.org/10.1038/ bjc.1986.122

Take Home Messages

HITRI plus
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- Immune system sees all! Immune suppression is the fundamental problem in cancer!
- RT has immunogenic potential to convert cold into hot environments!
- Immunogenic radiation features to be explored in terms of immunogenicity: RADIATION TYPE, DOSE, TIMING, VOLUME, IMMUNE DOSE, etc.
- Available experimental mouse data suggest CIRT might be ideal partner for IT when immunogenic potential is concerned.
- Clinical trials exploring potentially superior role of the particles/CIRT in combination with IT are required.

NIH U.S. National Library of Medicine
ClinicalTrials.gov

Immunotherapy and Carbon Ion Radiotherapy In Solid Cancers With Stable Disease (ICONIC)

ClinicalTrials.gov Identifier: NCT05229614

Recruitment Status : Recruiting
First Posted : February 8, 2022

CNAO National Center of Oncological Hadrontherapy

HITRI
Heavy Ion Therapy Research Integration

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