Intratumoral and Local Immunotherapy

Howard L. Kaufman
Immuneering Corp. and
Massachusetts General Hospital
Boston, MA

Immuneering

MGH 1811

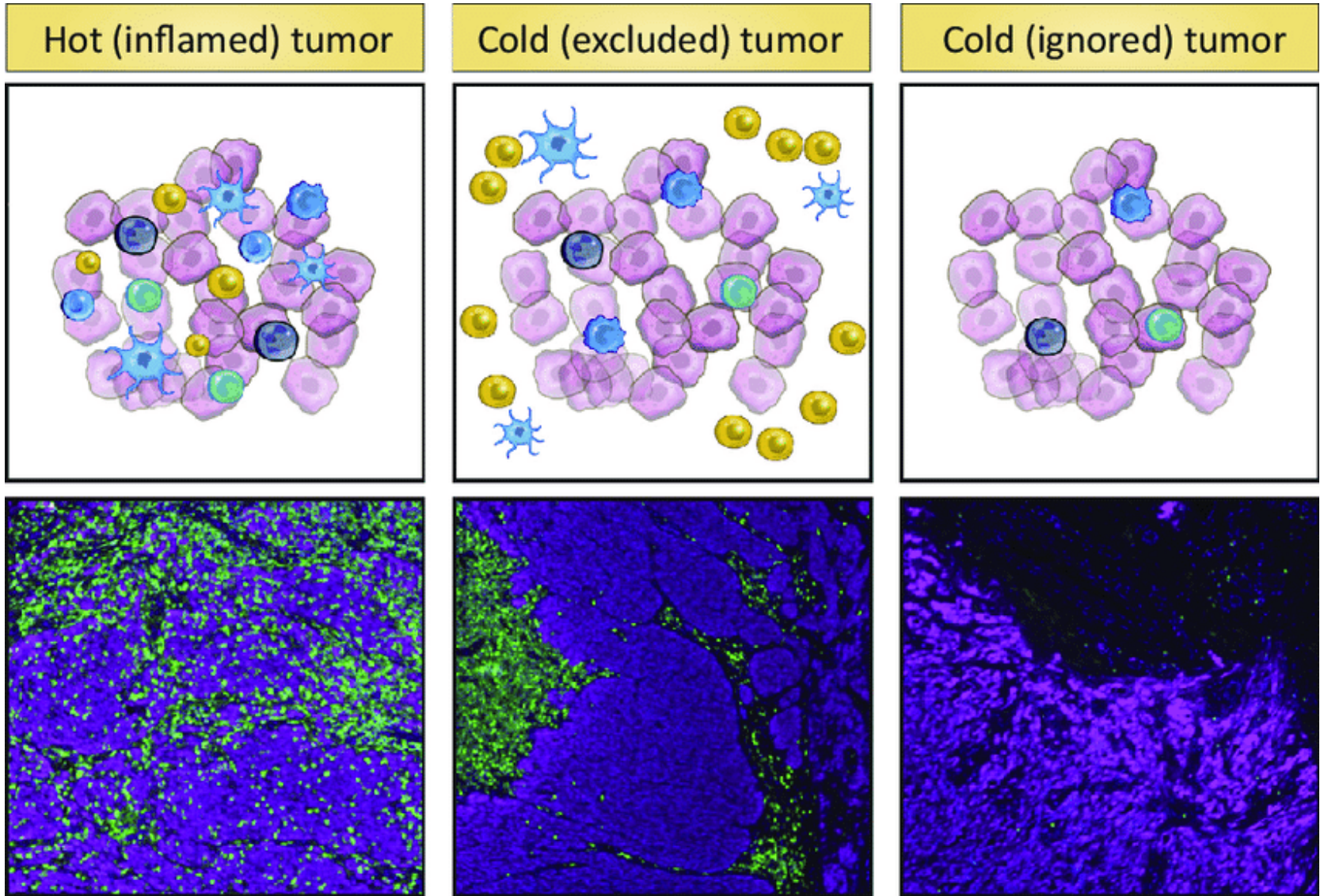
Disclosures

- I am an employee of Immuneering Corporation

Intratumoral Immunotherapy

Definitions and Rationale

Hot vs. cold tumor microenvironment



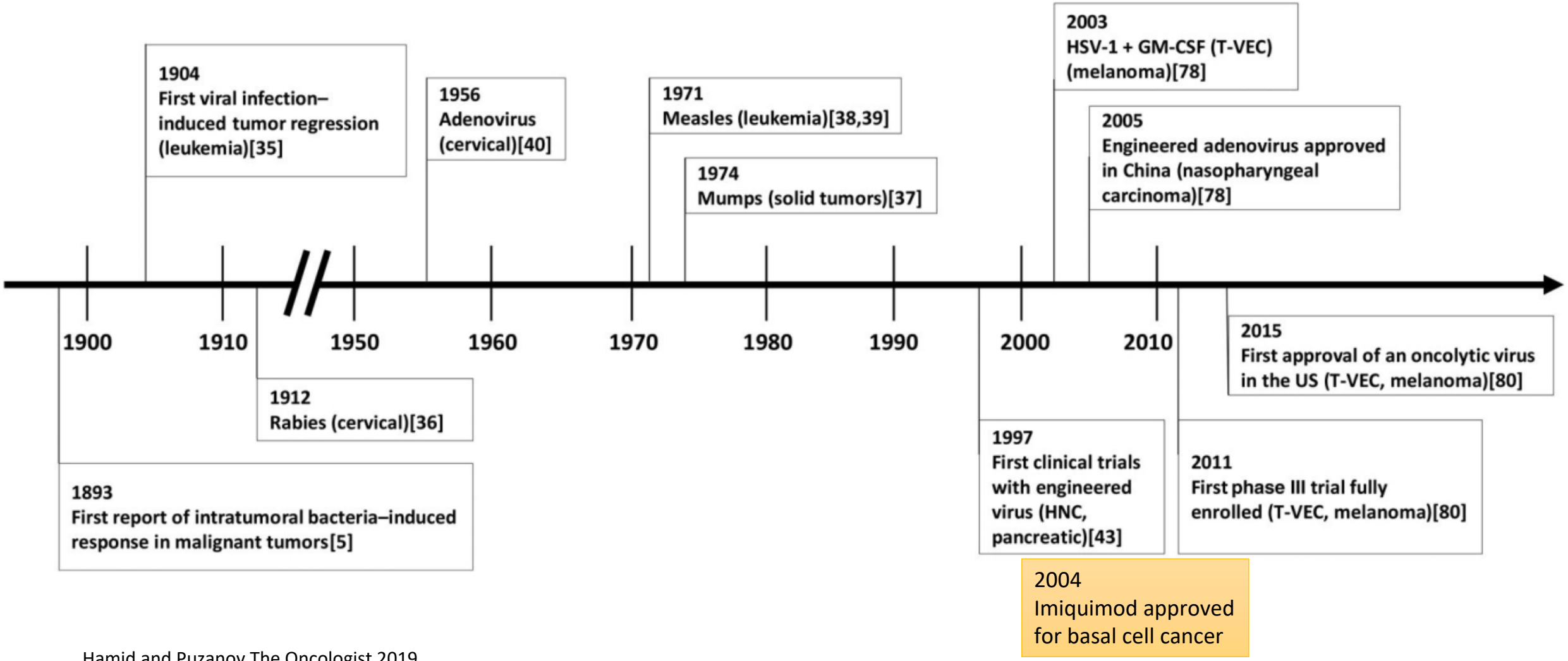
✓ A major goal of modern IO therapy is to establish Immune-inflamed (“hot”) tumor microenvironments

van der Woude et al. Trends Cancer 2017

What is intra-tumoral Immunotherapy?

- Therapeutic approach that delivers IO drugs directly into the tumor microenvironment
  - May be physical or chemical
  - Can be given by direct injection; or
  - Regional intra-vascular injection
  - Systemic delivery with local activation in the TME?
- Focuses on generating local immune responses
  - May also induce systemic immunity
- Expected to have a more favorable safety profile compared to systemic drug delivery

History of Intra-tumoral Therapy of Cancer

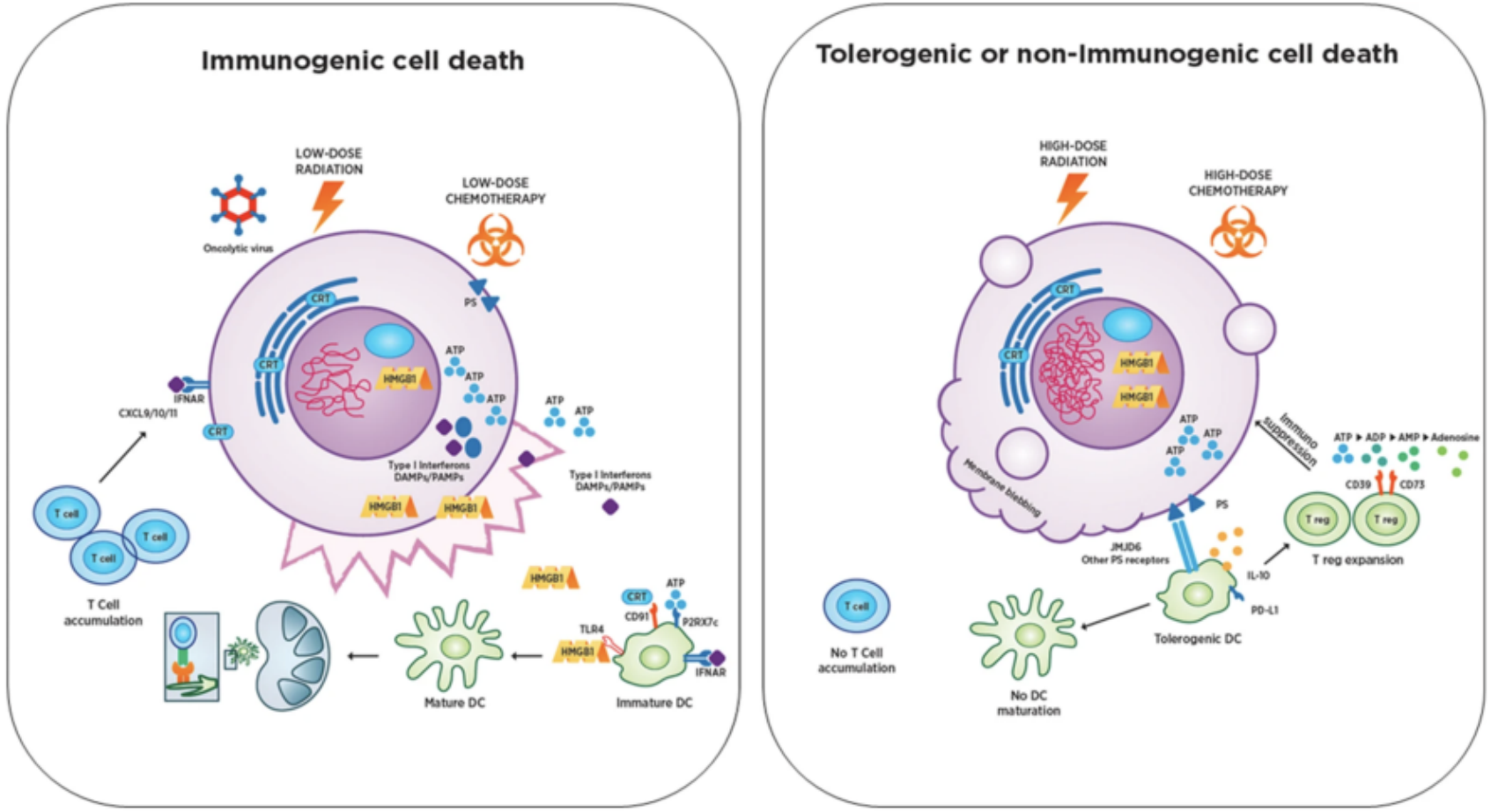


Hamid and Puzanov The Oncologist 2019

Intra-tumoral immunotherapy mediates anti-cancer activity through multiple mechanisms

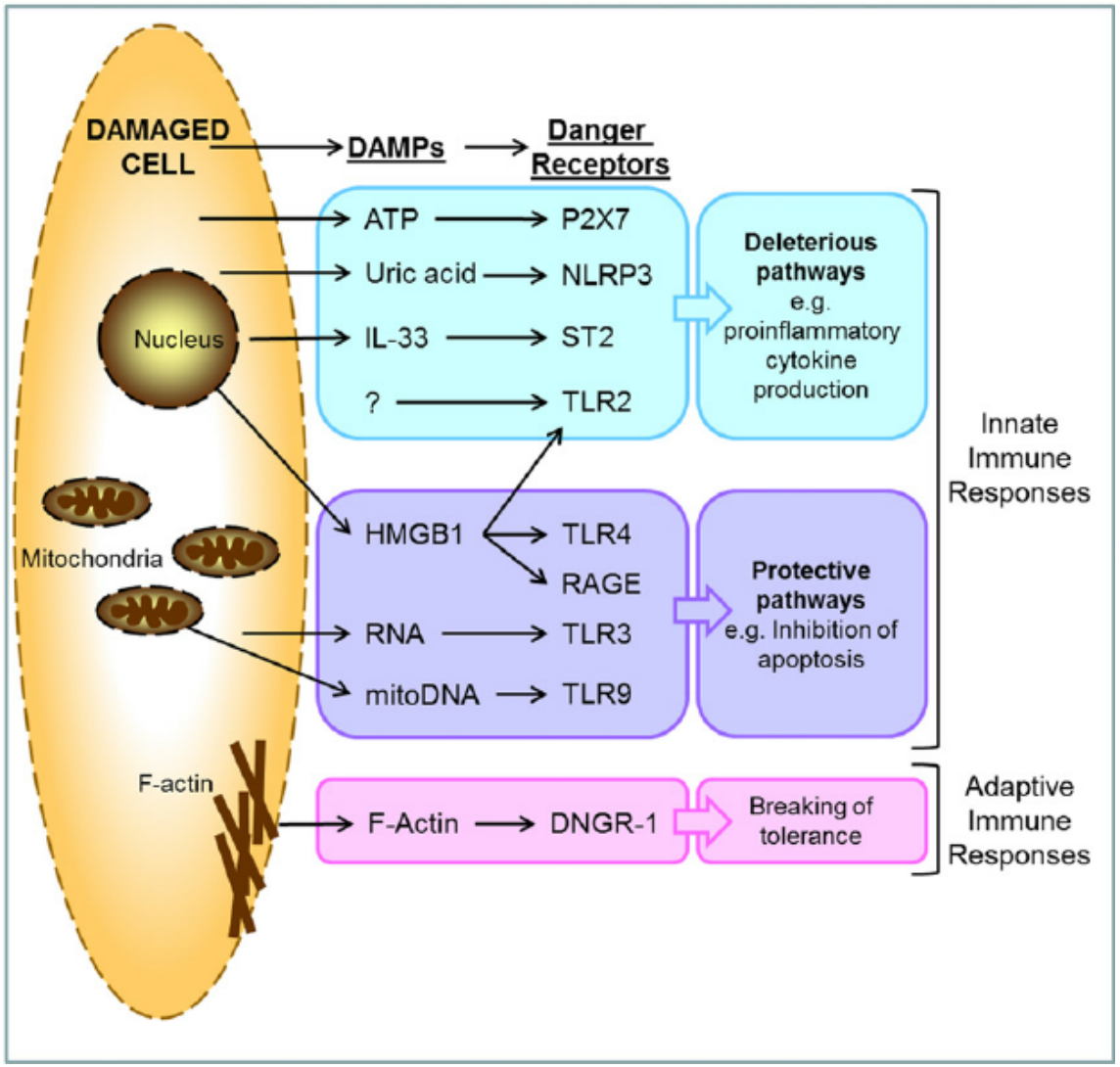
- Direct tumor cell cytotoxicity
  - May also impact other cells in the tumor microenvironment [1]
- Induction of host anti-tumor immunity
  - Local/regional immune responses [2]
  - Systemic (i.e., abscopal/anenestic) immune responses [3]

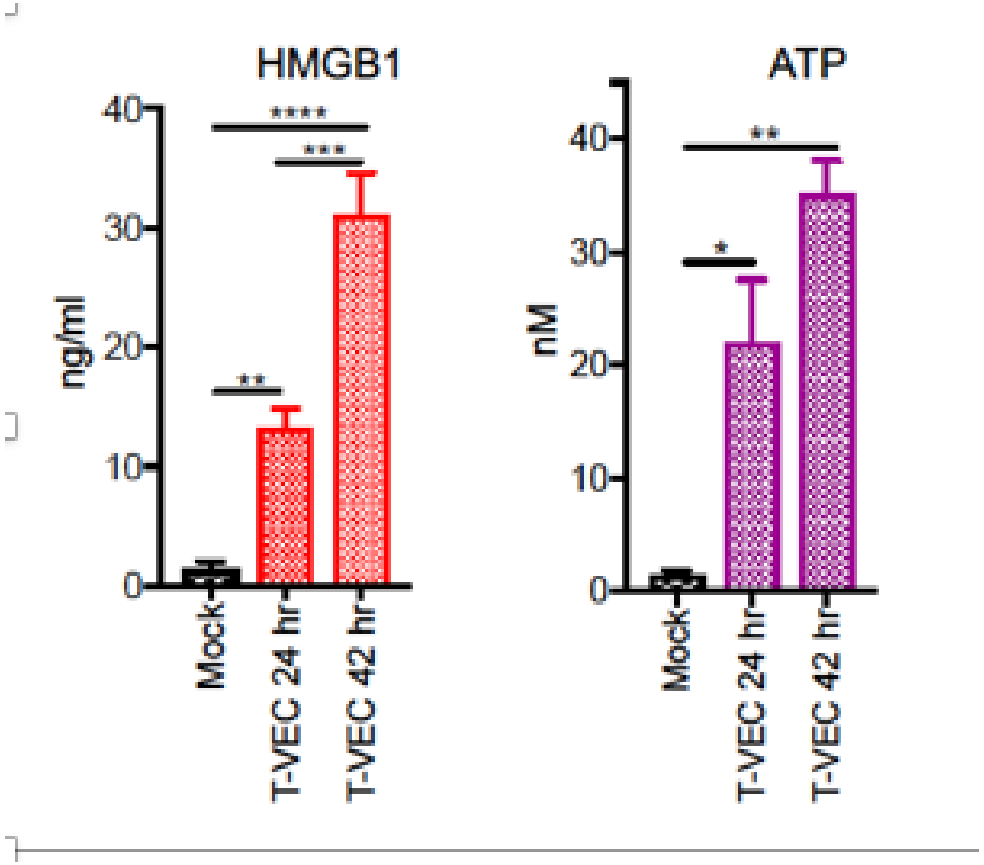
1. Immunogenic cell death



Bedognetti et al. JITC 2019

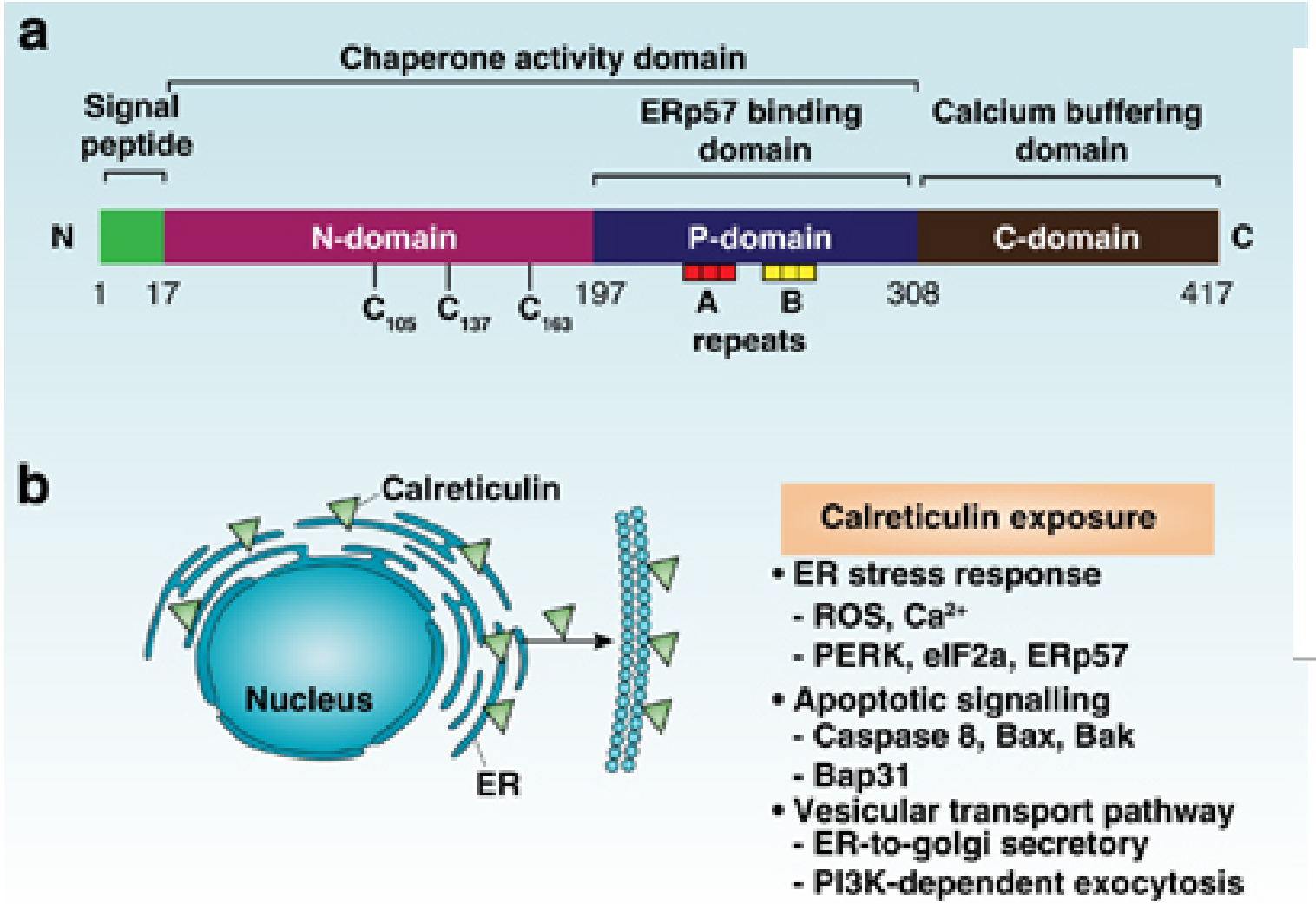
Traditional ICD measured by release of DAMPs

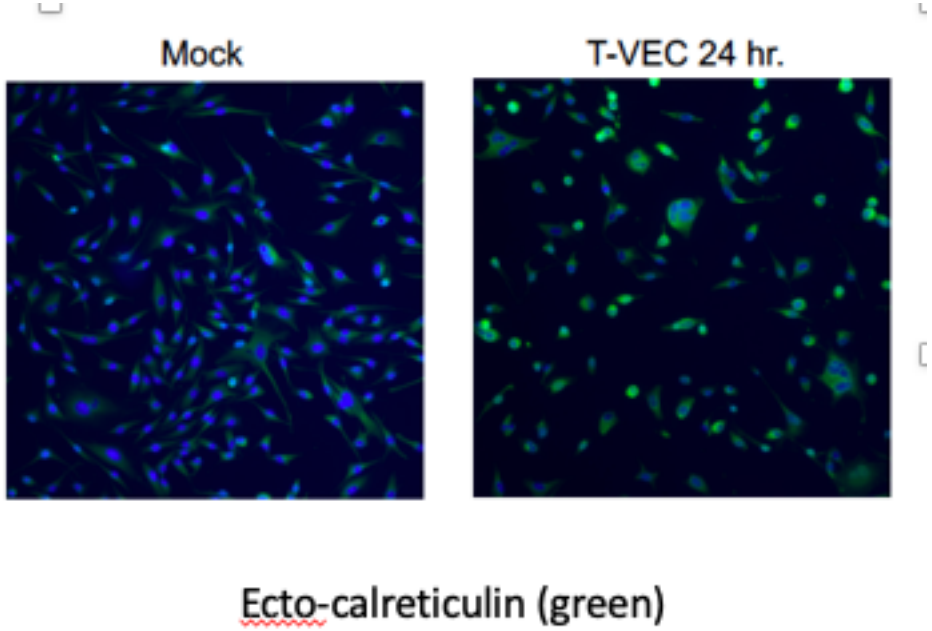




Courtesy Dr. Cory Hogaboam
Bommareddy et al. Oncolimmunol. 2018

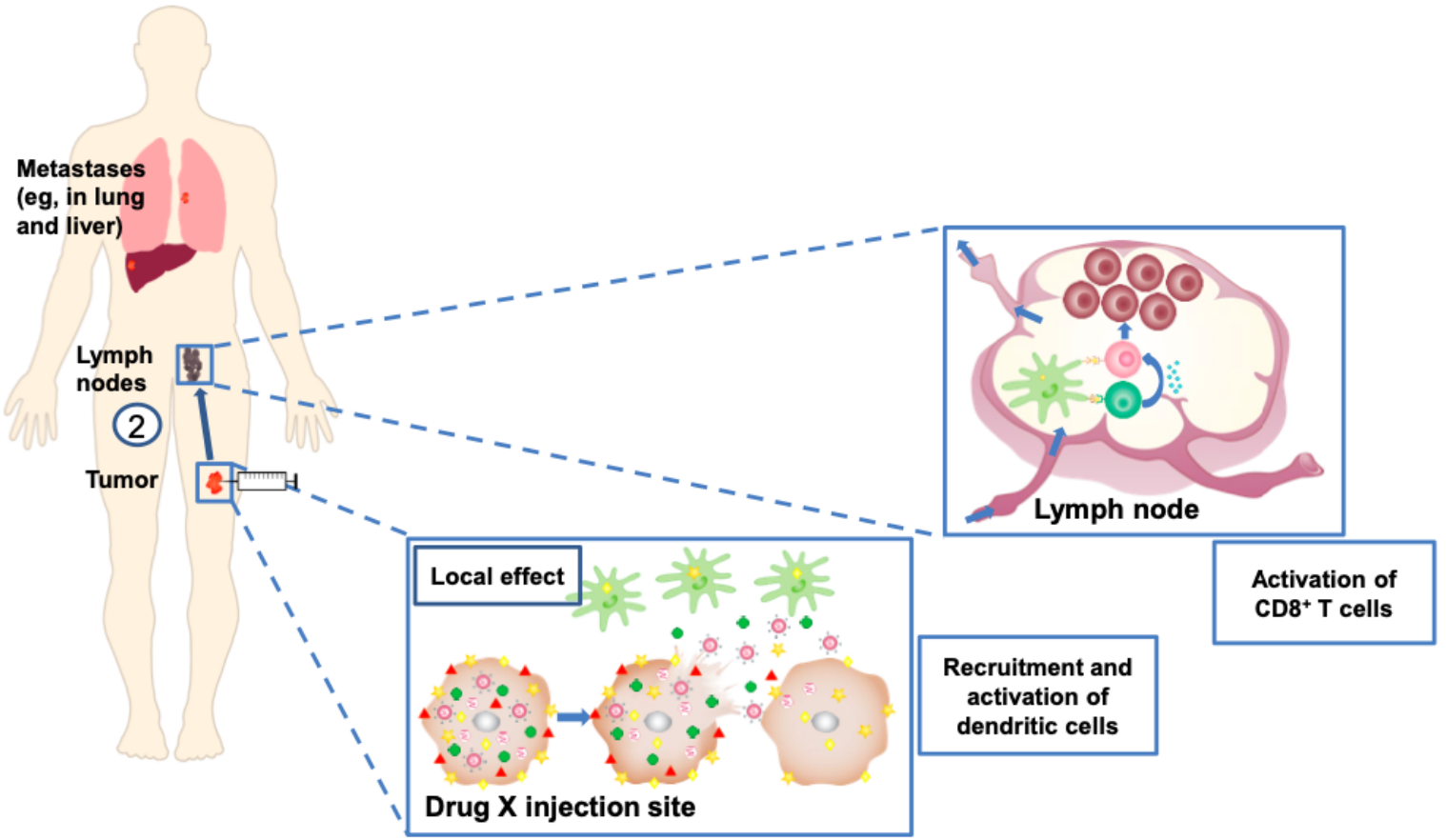
Ecto-calreticulin exposure denotes ICD





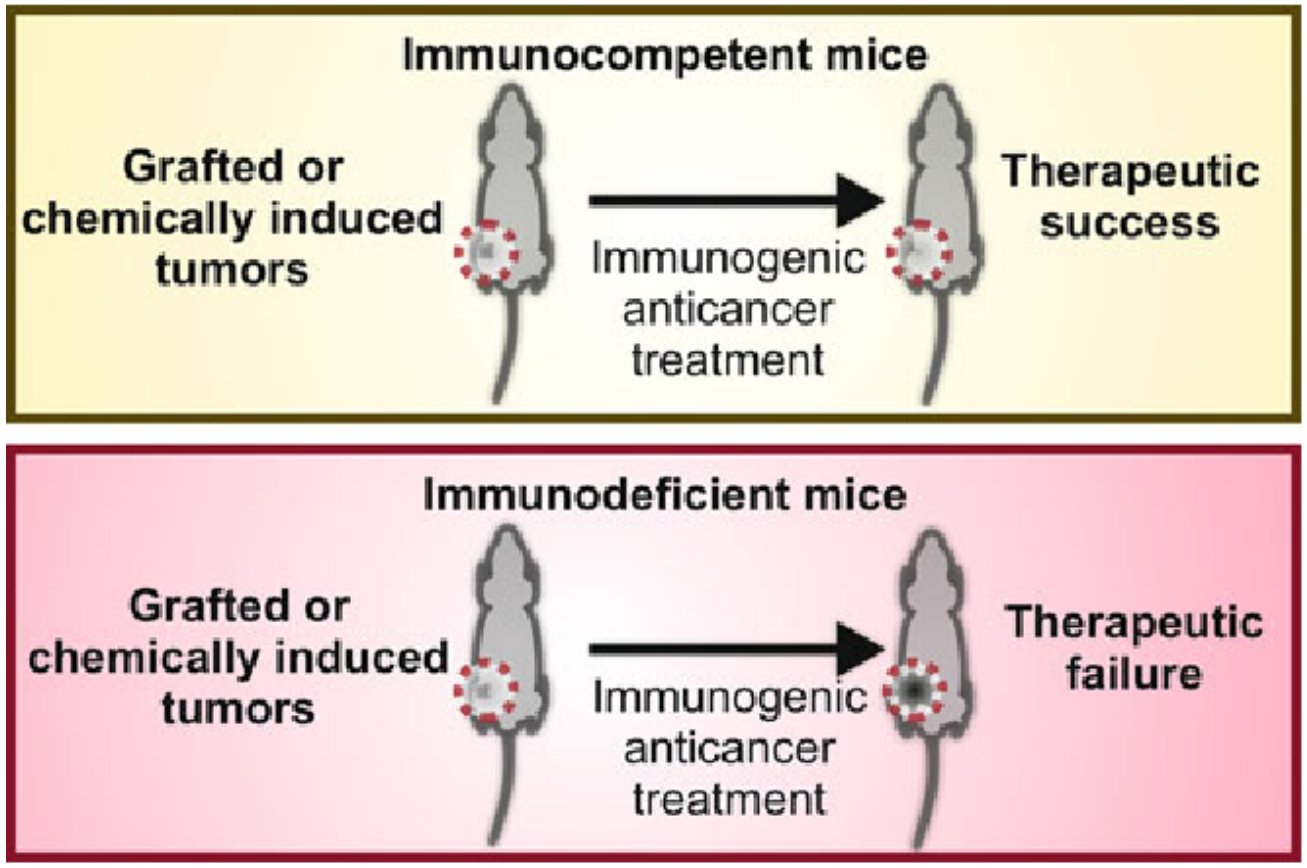
Hou et al. Cell Death Dis 2013
Bommareddy et al. Oncoimmunol. 2018

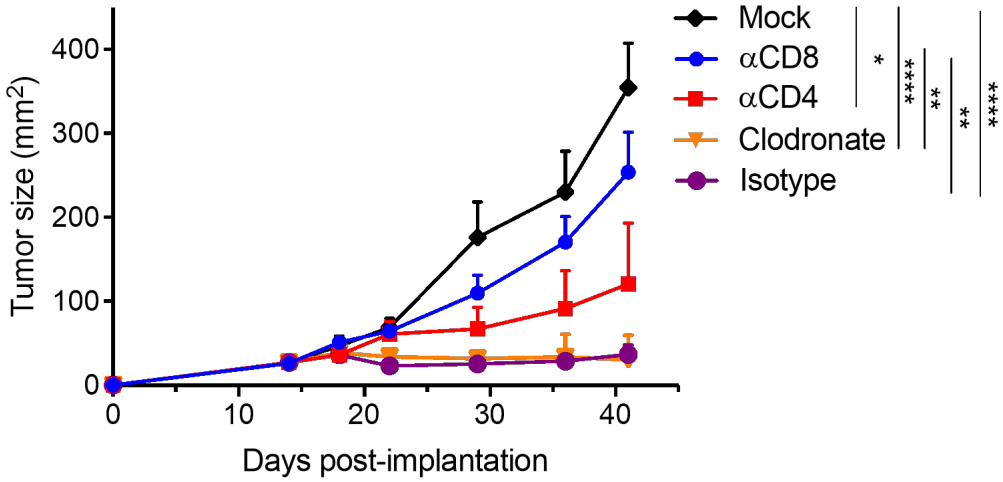
2. Intratumoral therapy promotes local and regional immune activation



oda M, et al. *Mol Ther*. 2000;2(4):324-239. Hawkins LK, et al. *Lancet Oncol*. 2002;3(1):17-26. Varghese S, et al. *Cancer Gene Ther*. 2002;9(12):967-978. Dranoff G. *Oncoge*
192. Liu BL, et al. *Gene Ther*. 2003;10(4):292-303. Eager R, et al. *Mol Ther*. 2005;12(1):18-27. Hu JC, et al. *Clin Cancer Res*. 2006;12(22):6737-6747. Fukuhara H, et al. *Curr*

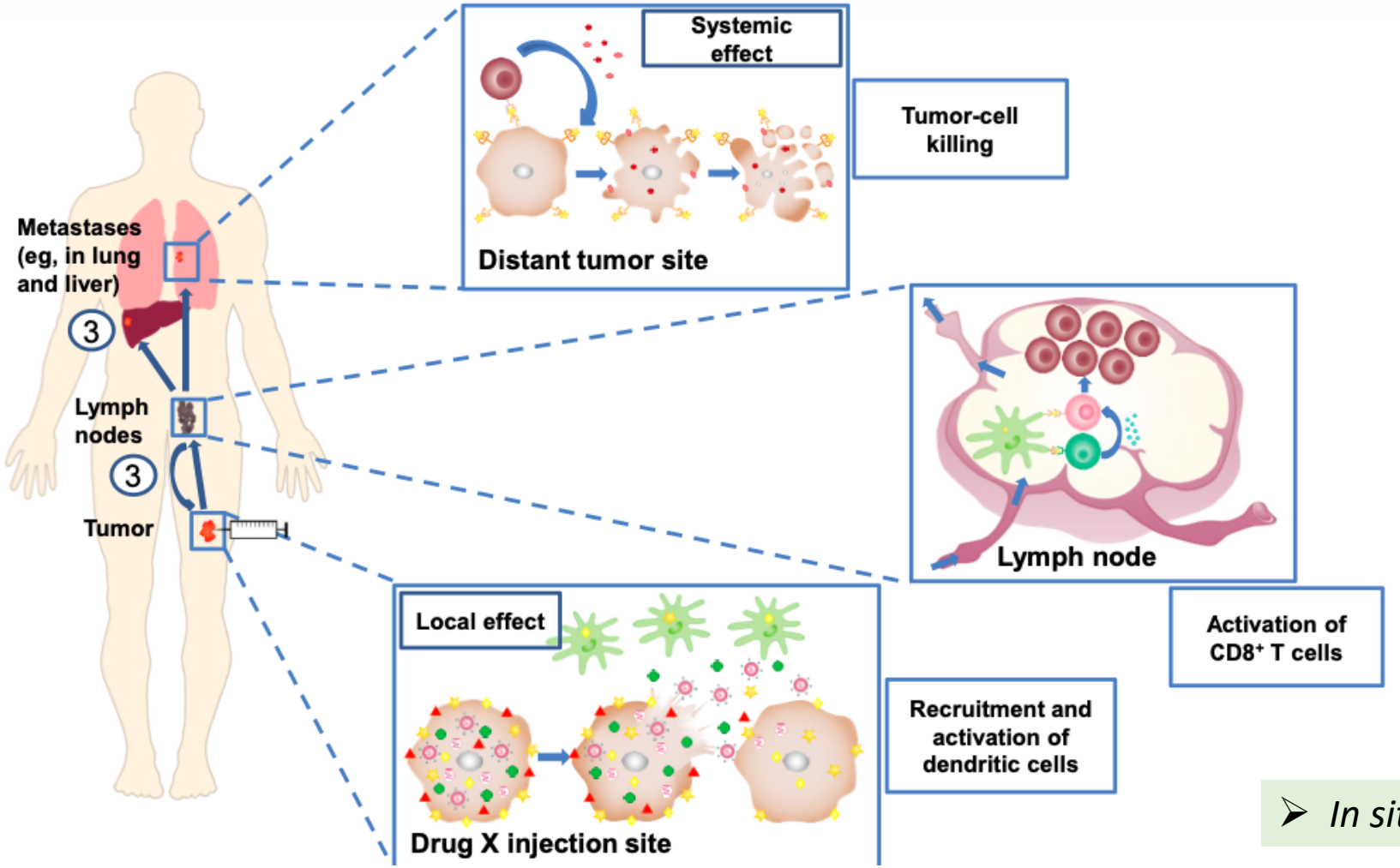
Pre-clinical strategies for demonstrating immunity with local immunotherapy agents





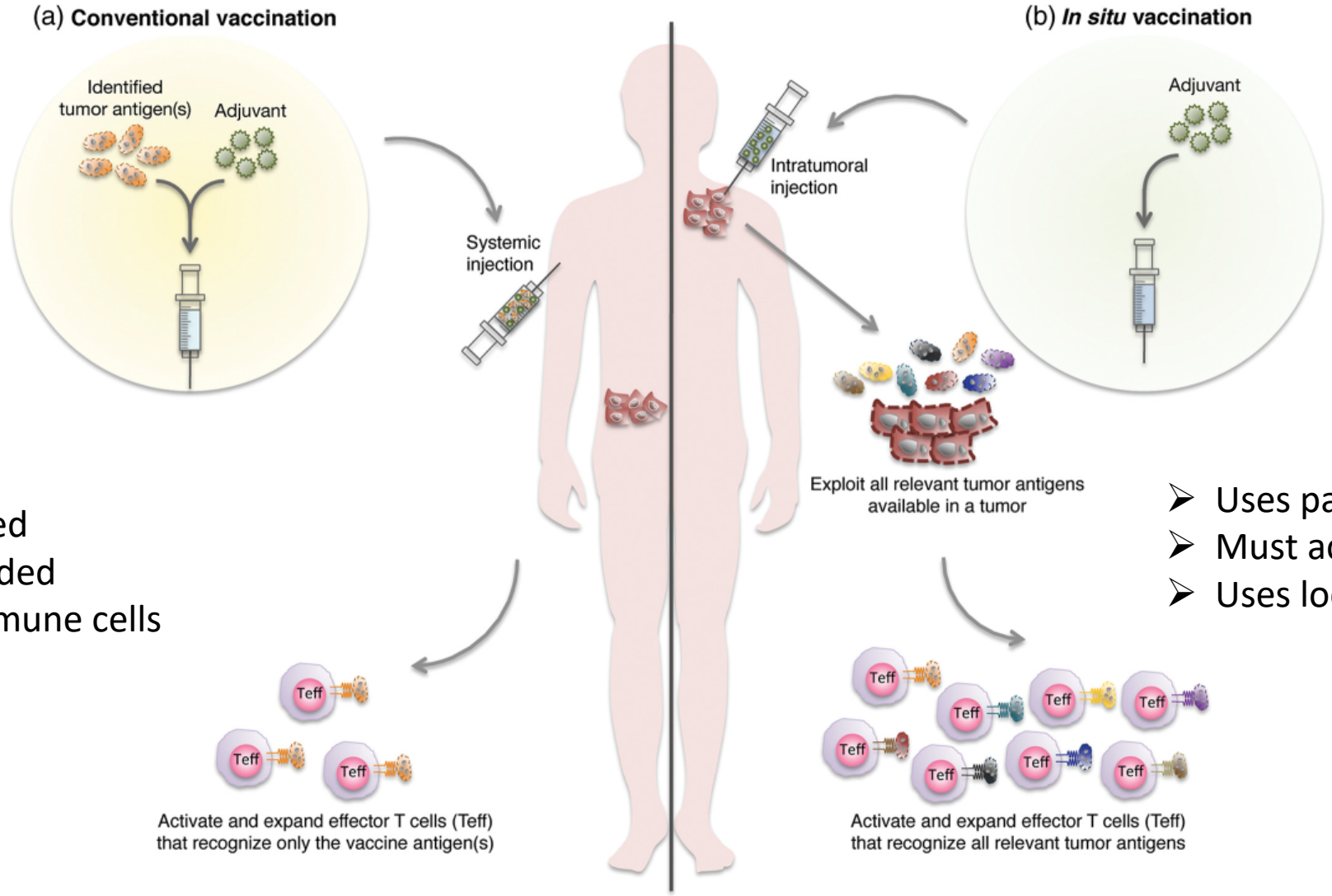
Kepp, Galluzzi, et al. Cancer Metastasis Rev 2011
Bommareddy et al. Science Transl Med 2018

3. Intratumoral therapy ***may*** induce systemic immunity (i.e., abscopal or anenestic effect)



- *In situ* "vaccination" effect

Intratumoral immunotherapy may have an *in situ* vaccination effect

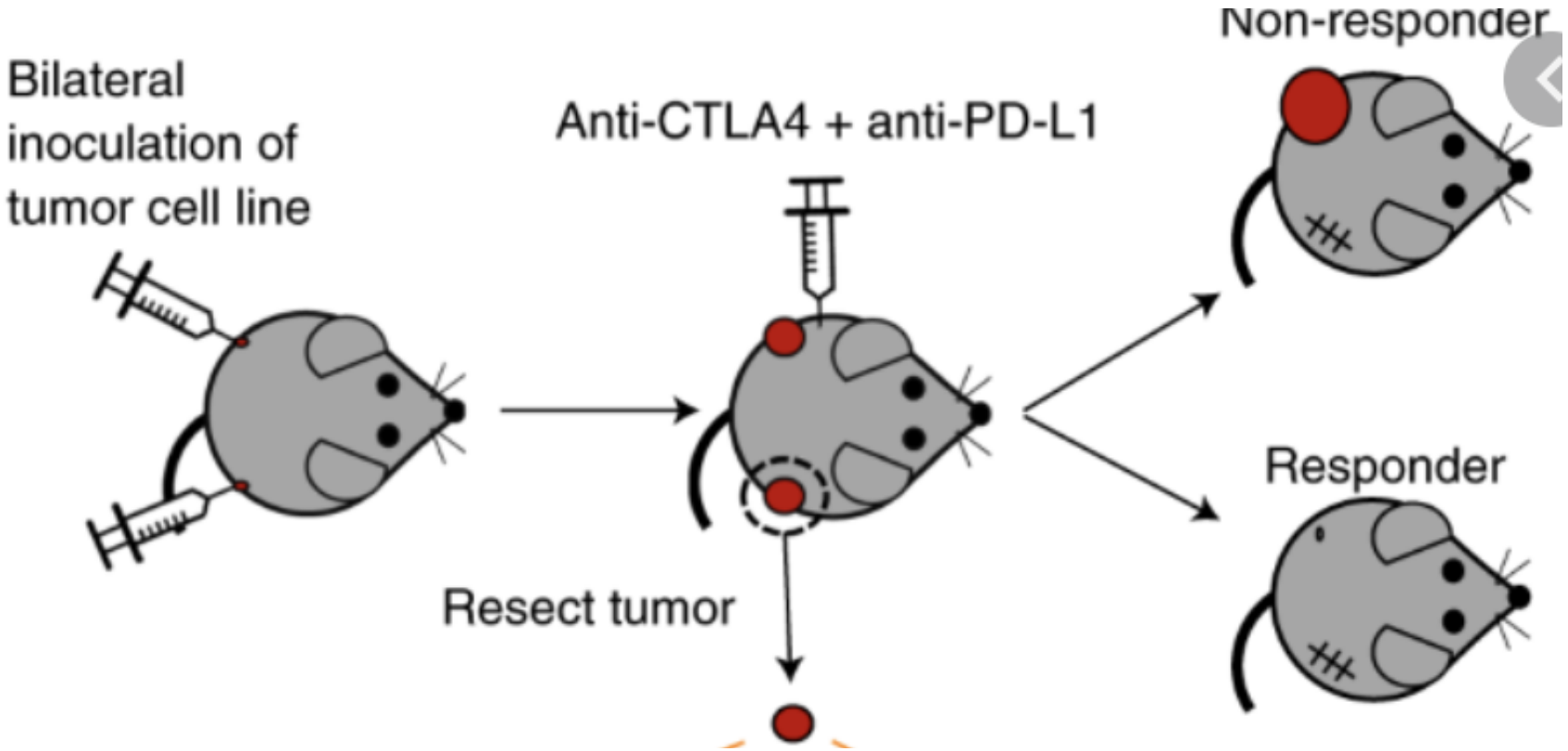


- Antigens defined
- Tumor not needed
- Use normal immune cells

- Uses patient tumor neoantigens
- Must access tumor
- Uses local immune system

Sheen and Fiering WIREs 2018

Bilateral flank tumor model to assess systemic anti-tumor activity with local immunotherapy



Zemeck et al. Nature Protocols 2020

Benefits of Intra-tumoral Immunotherapy

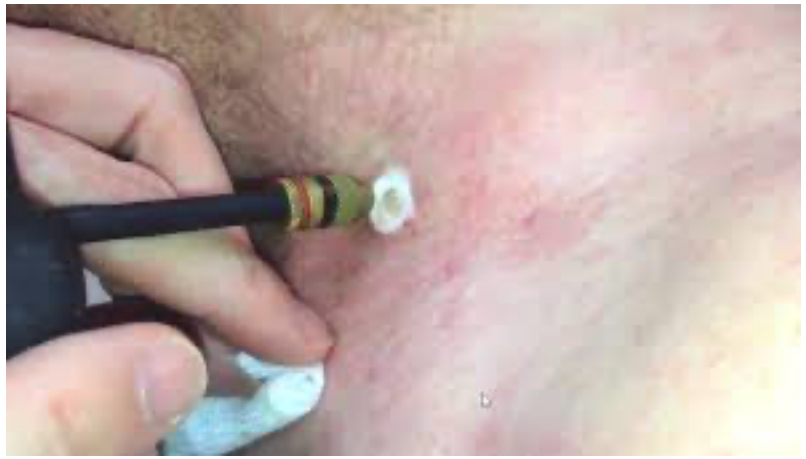
- Allows direct access to multiple cells in the tumor microenvironment
- Able to use established tumor features (e.g., in situ vaccine effect)
- No need to identify tumor-associated antigens
- Generally, has been associated with limited toxicity
- Easy to promote serial biopsy and biomarker analyses

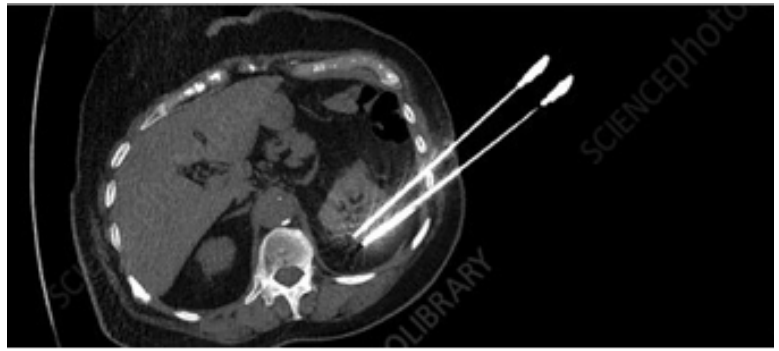
Intratumoral Immunotherapy

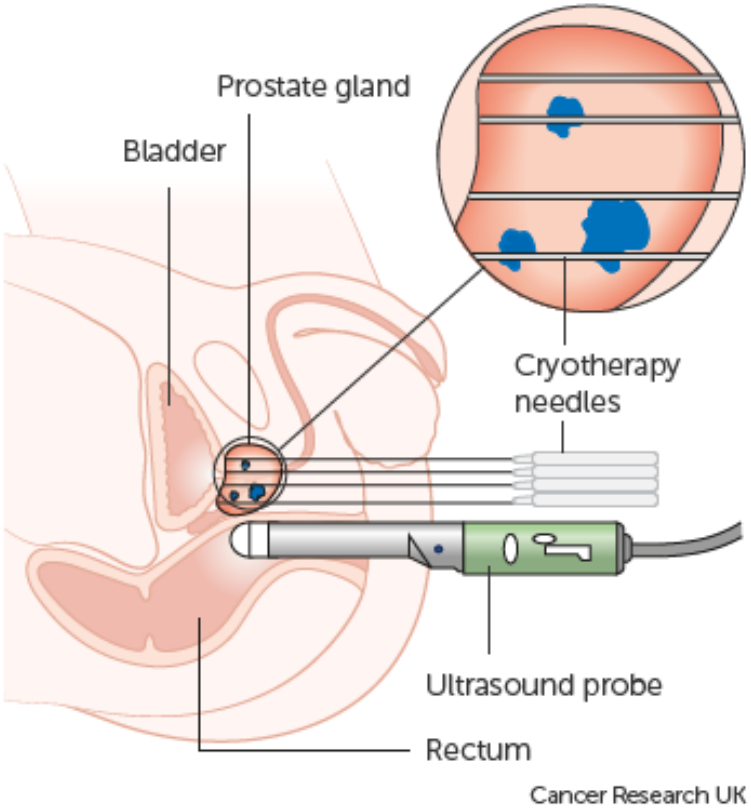
Types of Intratumoral Therapy

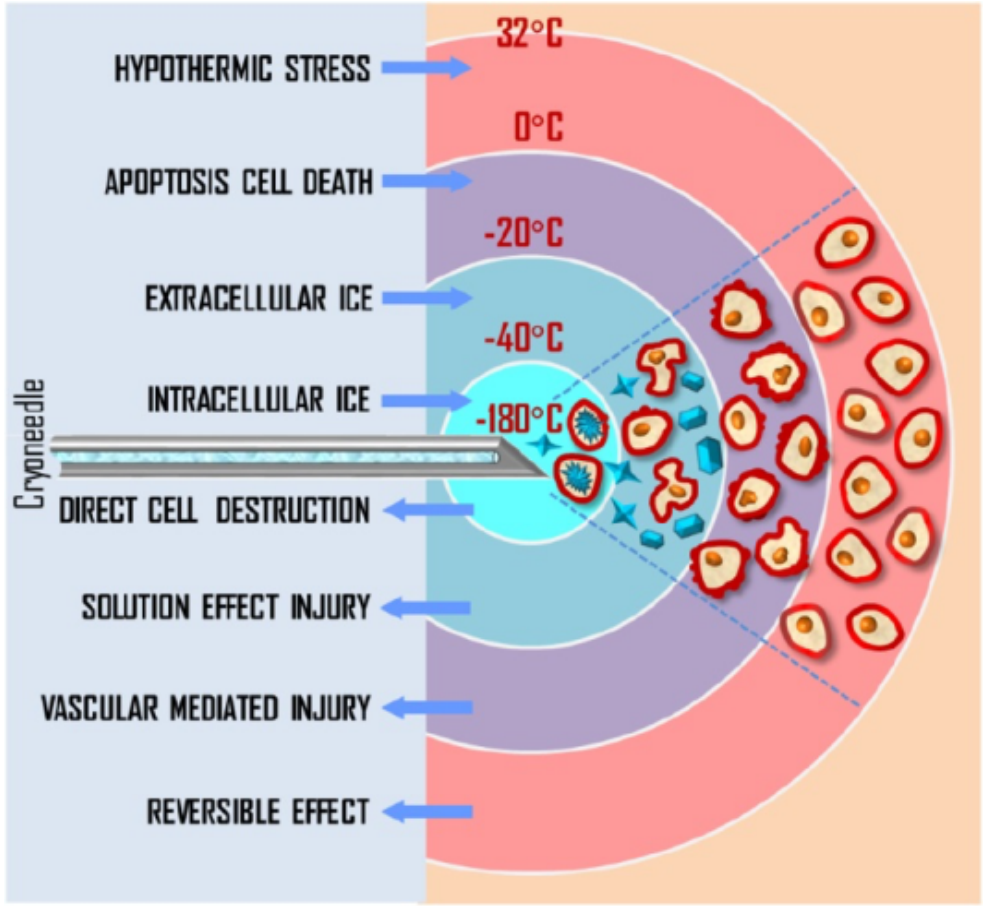
Physical Intratumoral Therapy

Cryotherapy





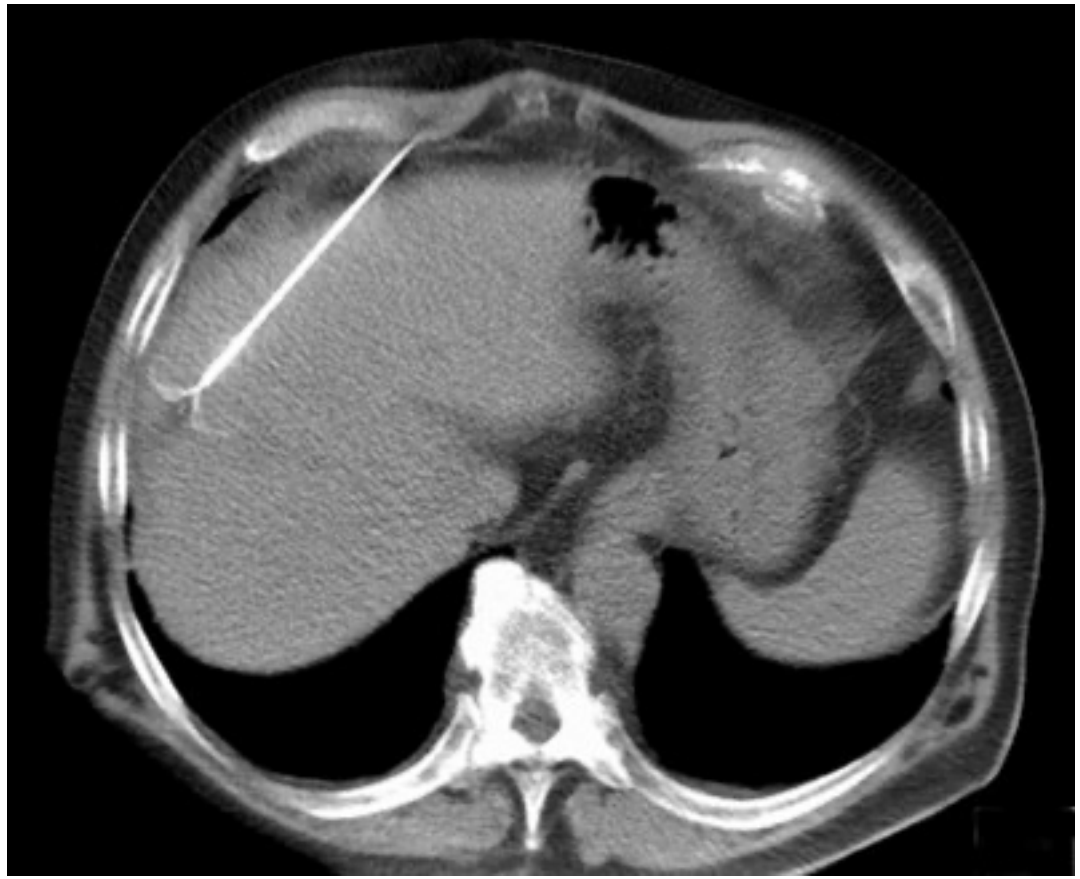


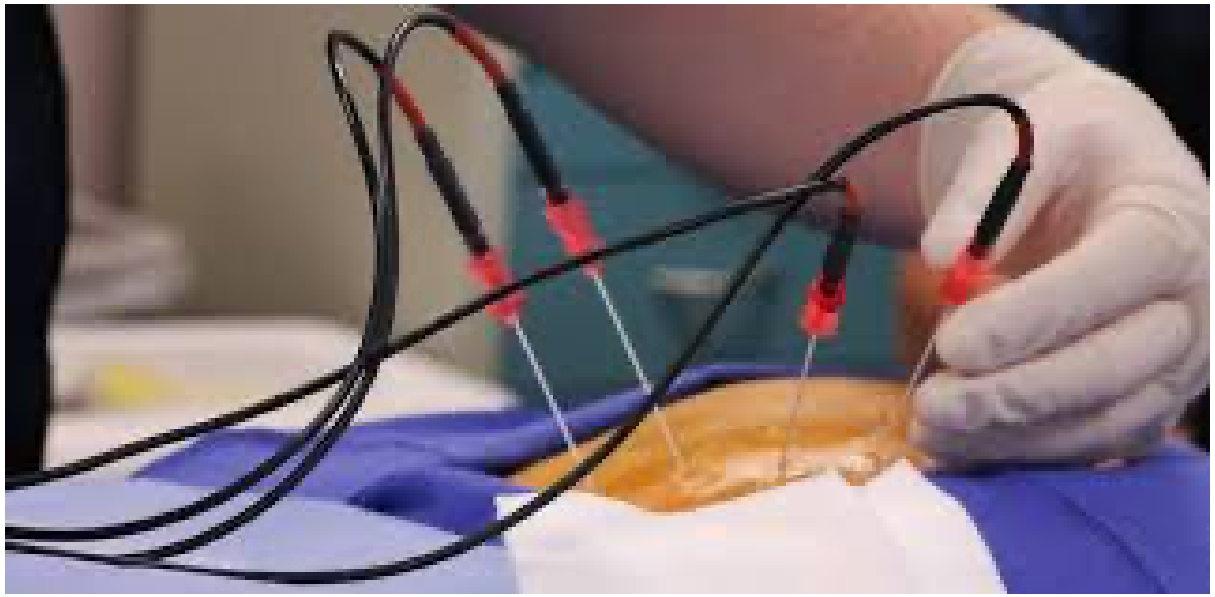


Toxicity:

- Pain
- Hemorrhage
- Edema
- Numbness
- Neuropathy
- Alopecia

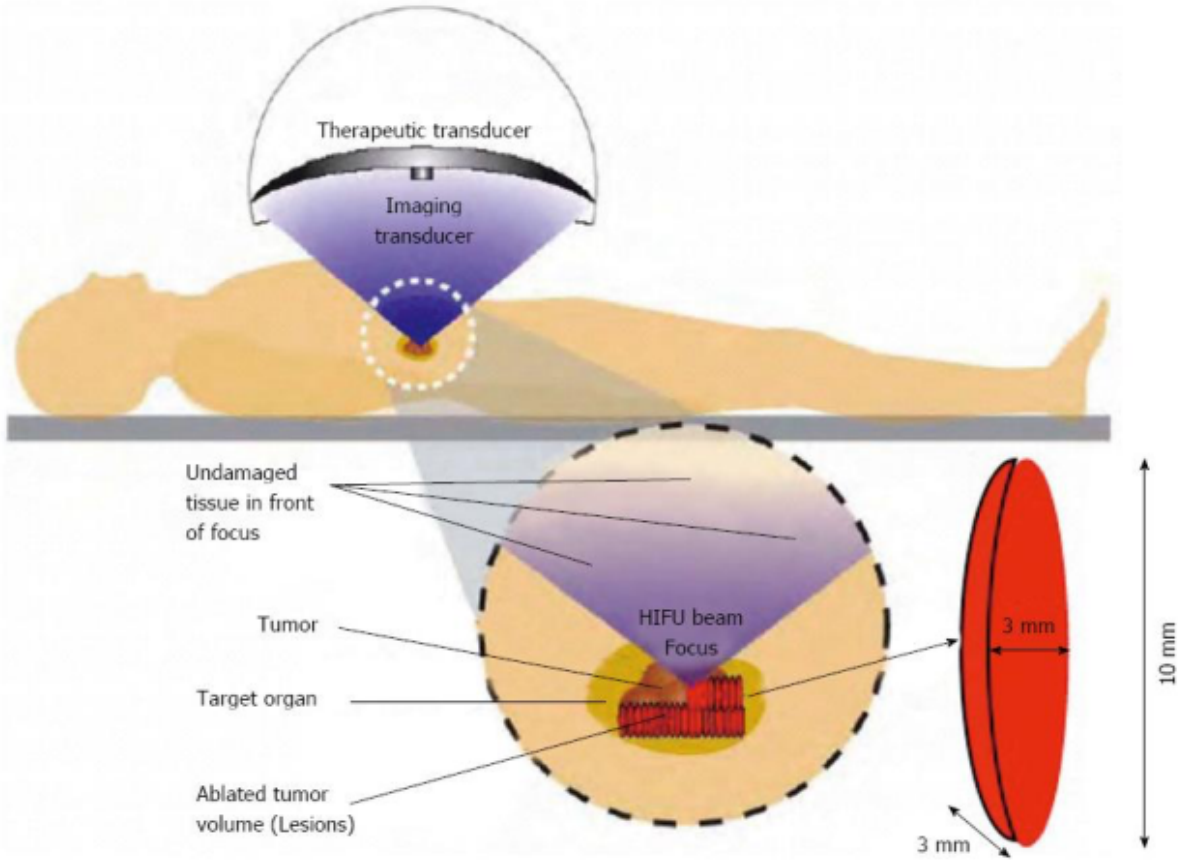
Microwave and Radiofrequency Ablation





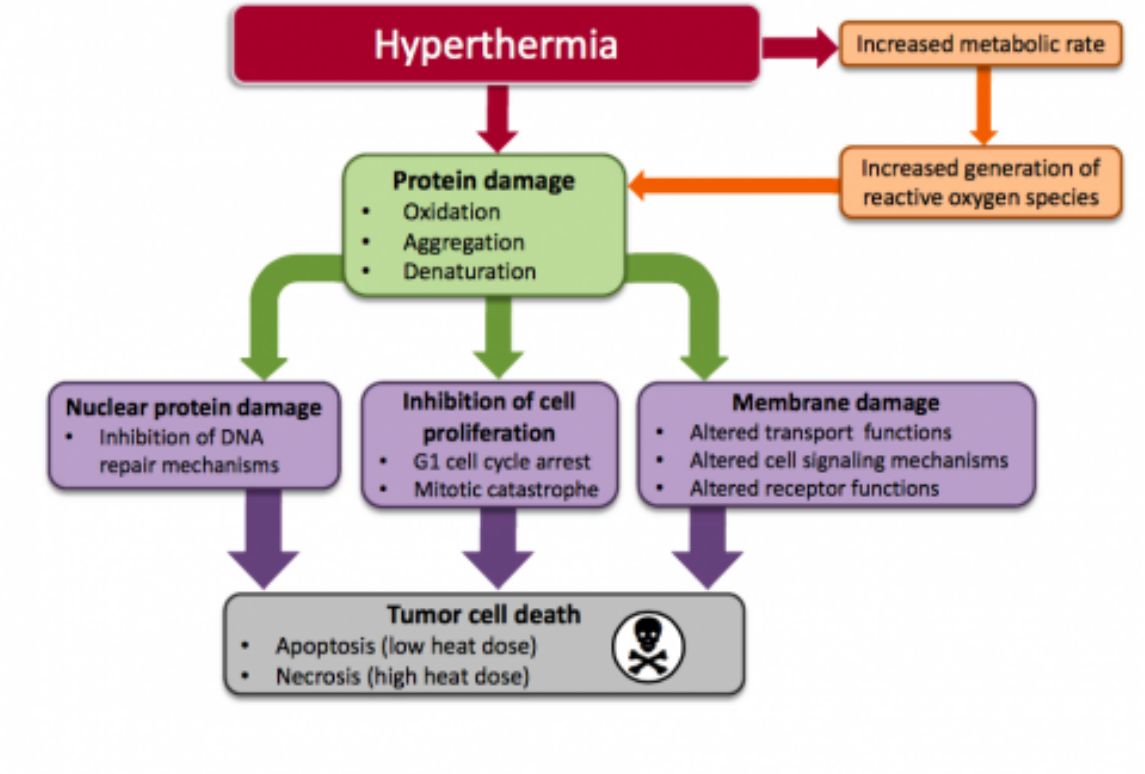
- Tumor entered with thin needle and probe
- Apply electrical current (radiofrequency) or microwave energy
- Tumor necrosis induced
- Residual scar left behind

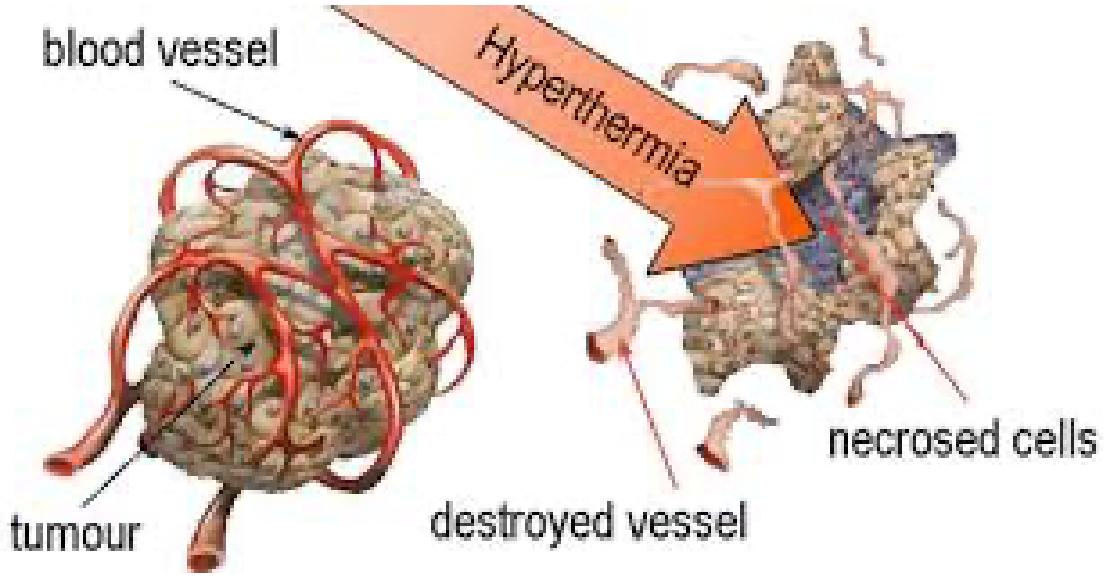
High-intensity Focused Ultrasound



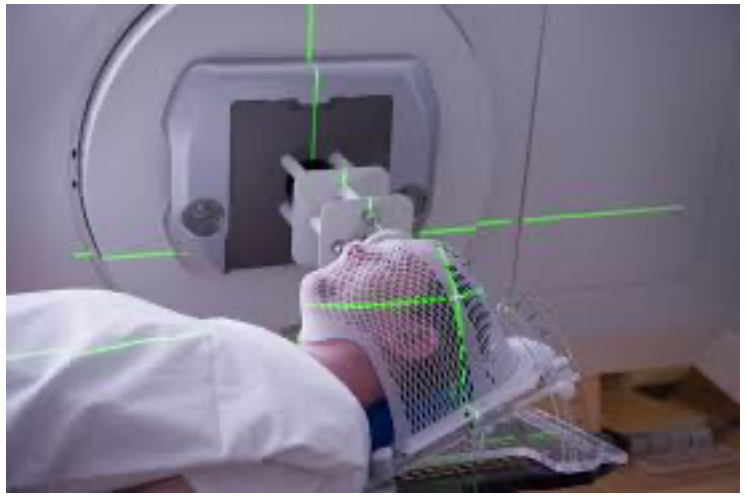
- Non-invasive therapeutic technique
- Uses lower frequency and continuous waves
- Induces thermal damage in tissue (65-85 °C)
- Pulsed waves induce mechanical damage
- Can use with ultrasound or MRI imaging
- HIFU approved in U.S. for prostate cancer treatment in 2015
- Many other tumors under study

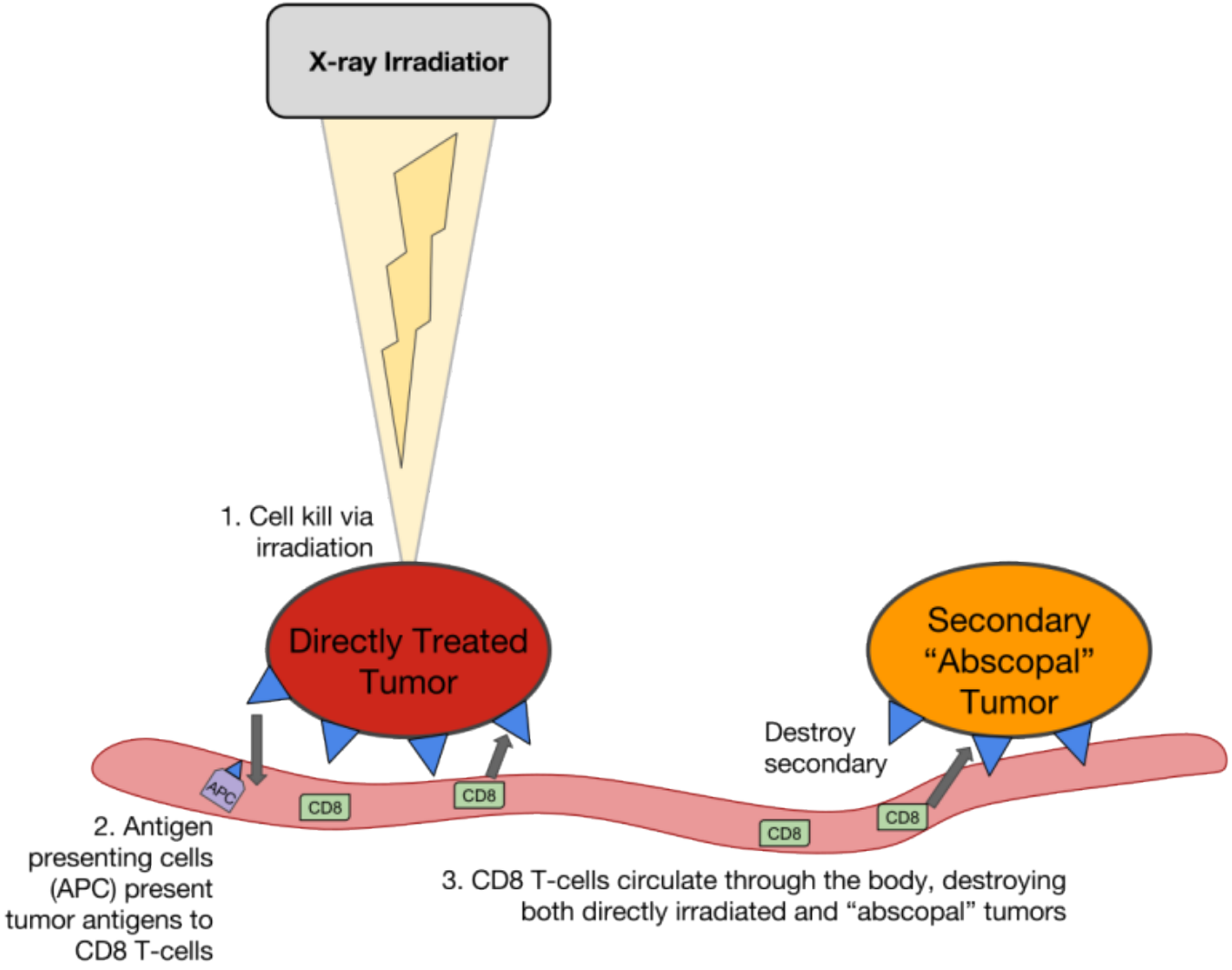
How does hyperthermia mediate anti-tumor activity?



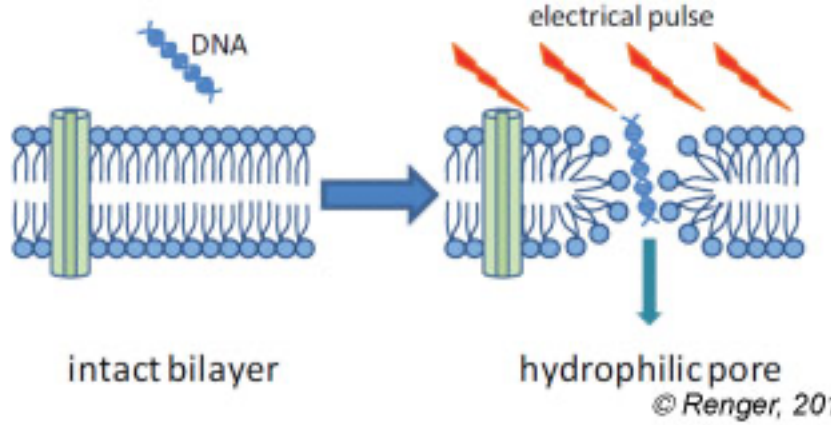


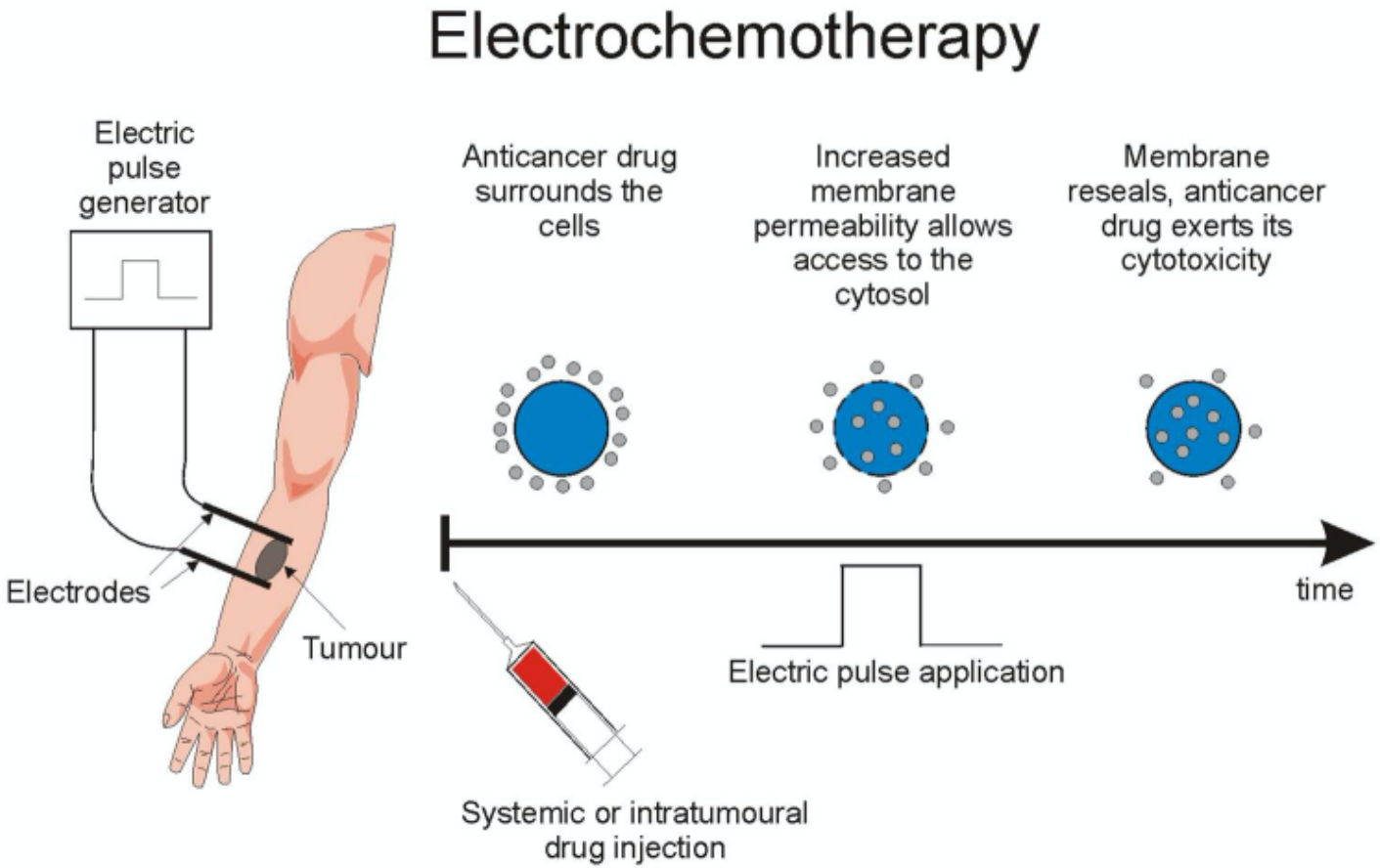
Radiation Therapy

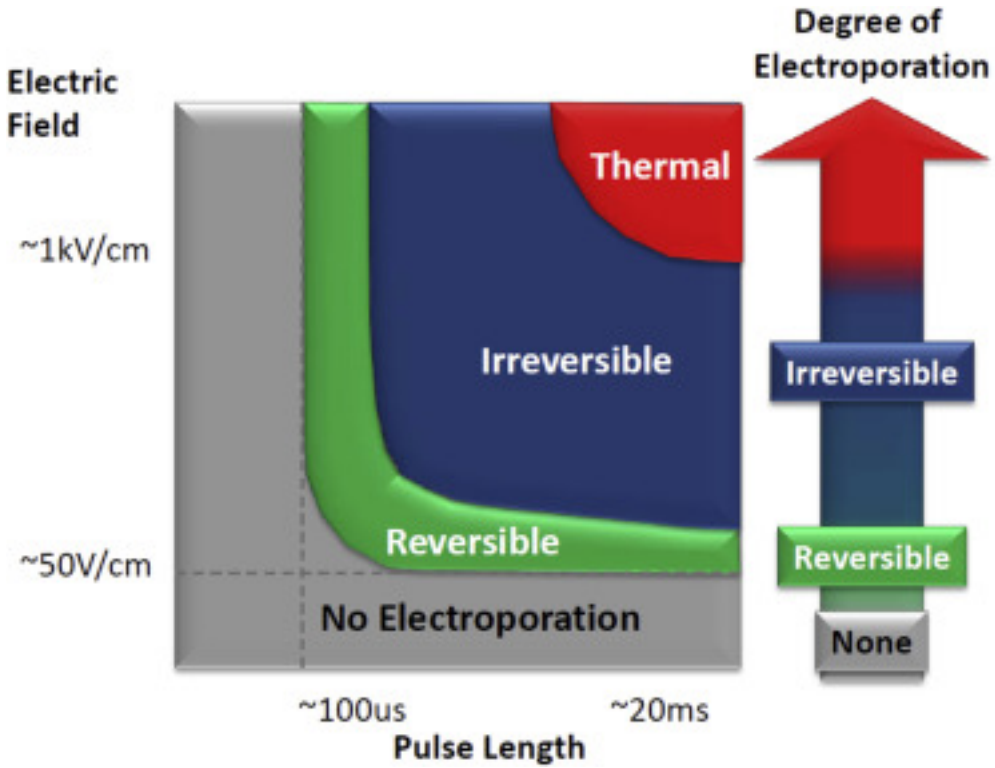




Electroporation

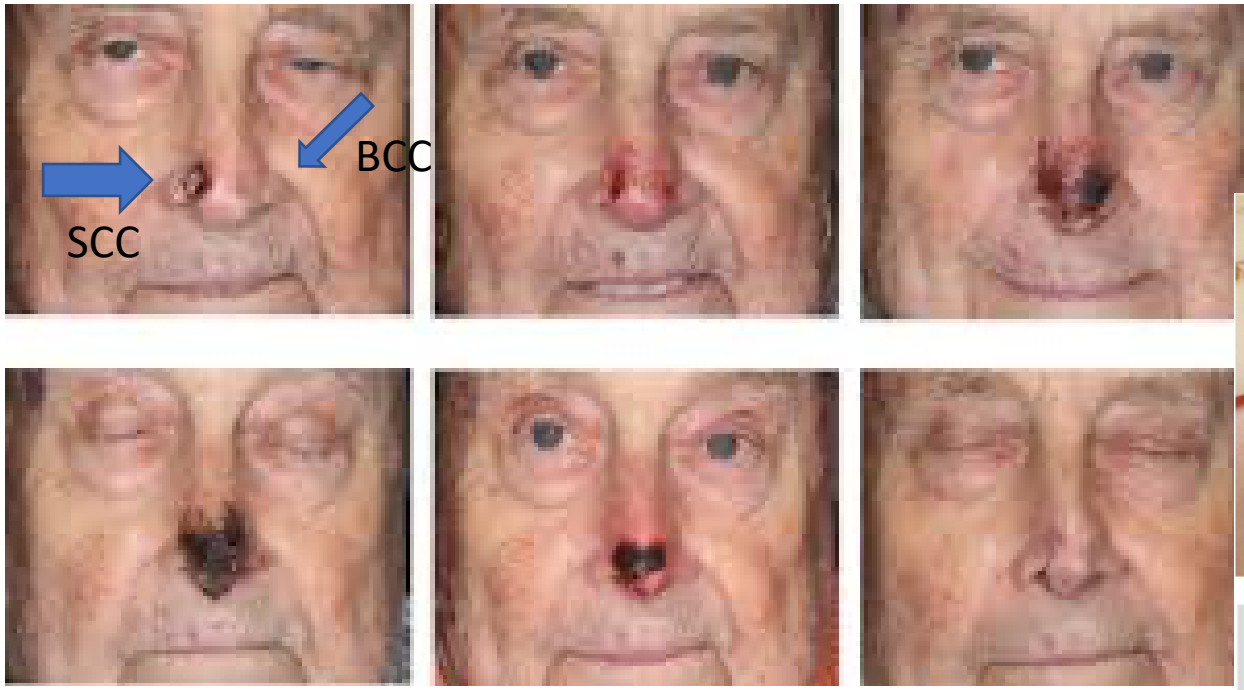






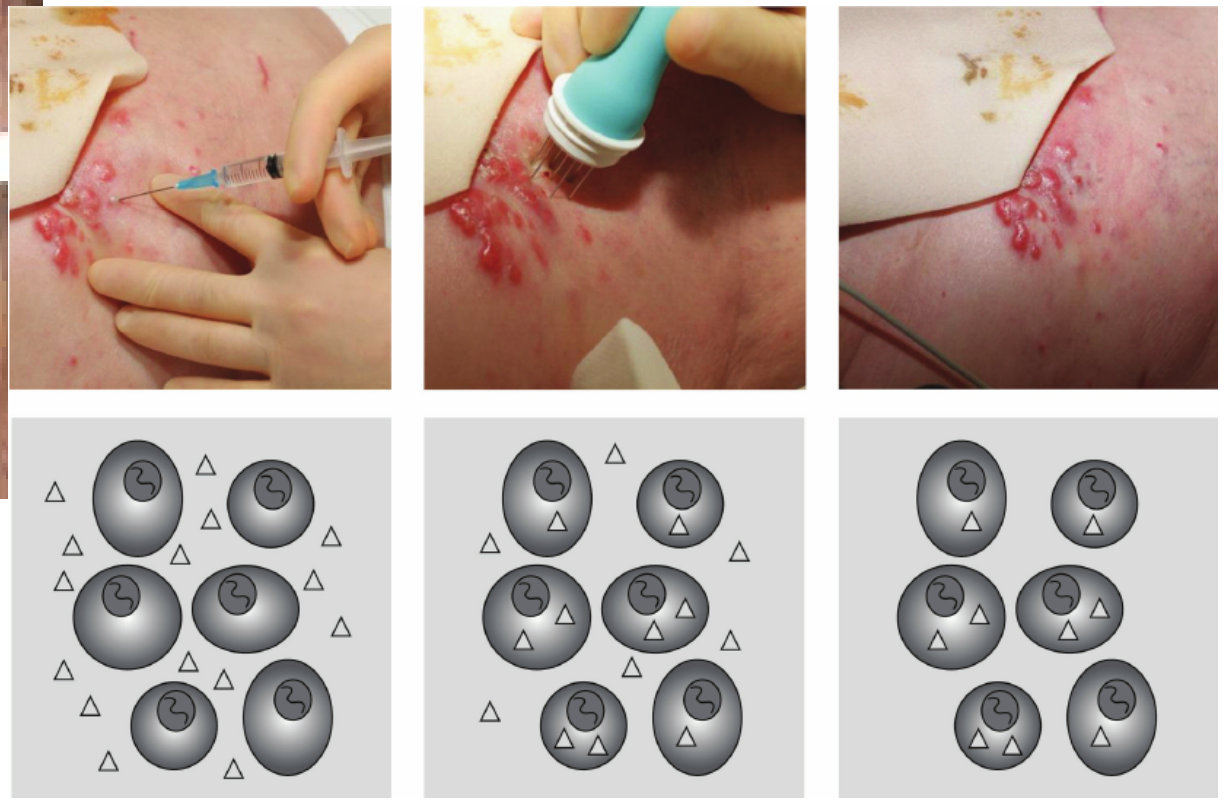
Drug-related Intratumoral Therapy

Intratumoral chemotherapy and electrochemotherapy



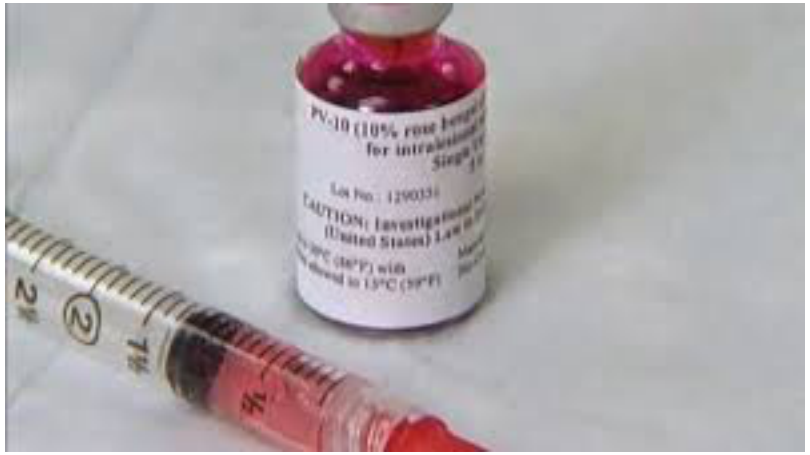
Treated with six weekly intra-lesional injections of 5-FU

Courtesy Julie Gehl



Electrochemotherapy with bleomycin

PV-10 in melanoma



Overall best response	First treatment	Second treatment	Third treatment	Fourth treatment
Complete response	13	8	3	1
Partial response	24	12	3	-
Stable disease	3	4	1	-
Progressive disease	5	5	-	-
Total	45	29	7	1

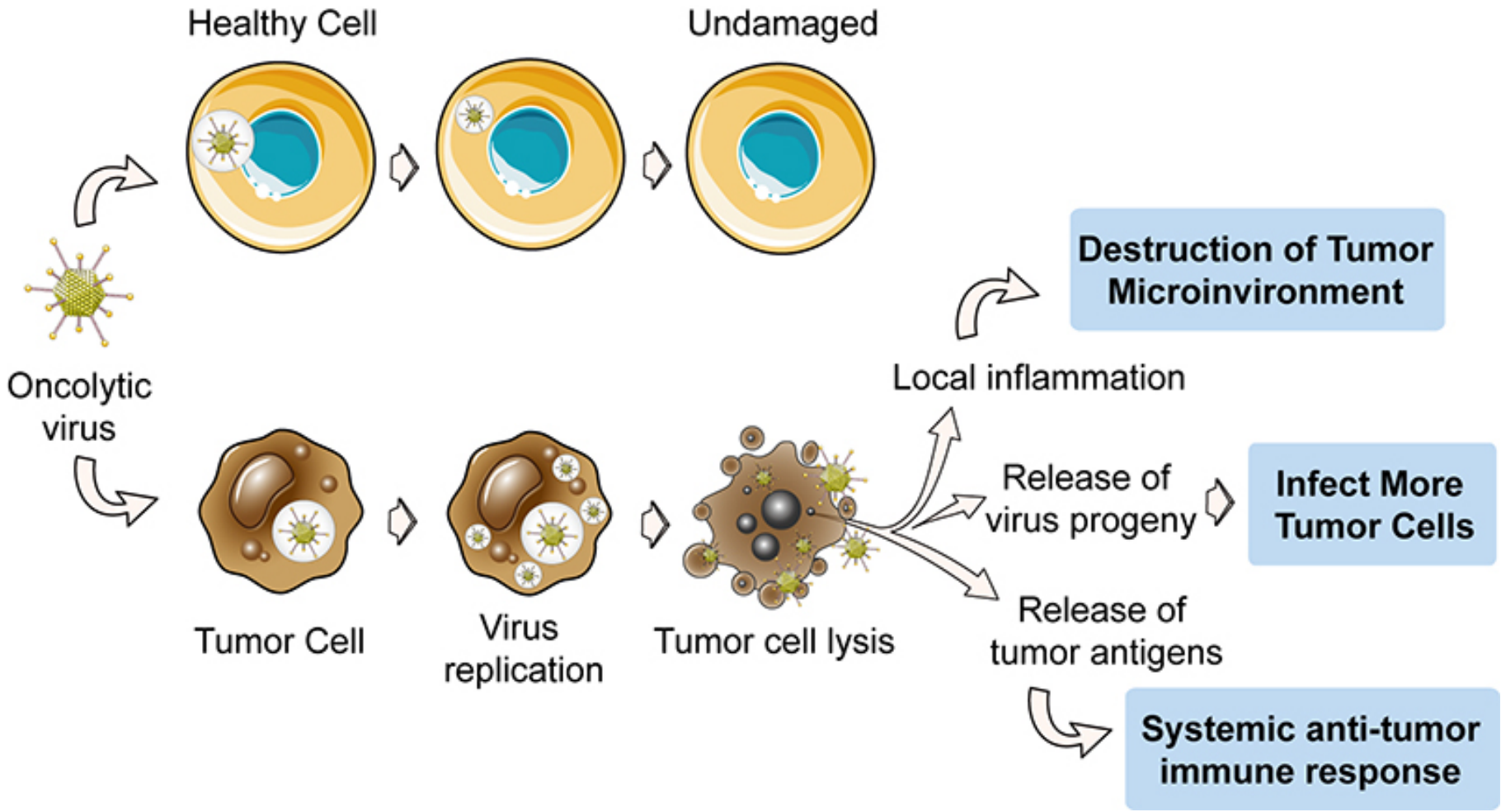
In-transit mets
45 patients

- 87% ORR
- 42% CR

Read et al. J Surg Oncol 2018

Oncolytic Viruses

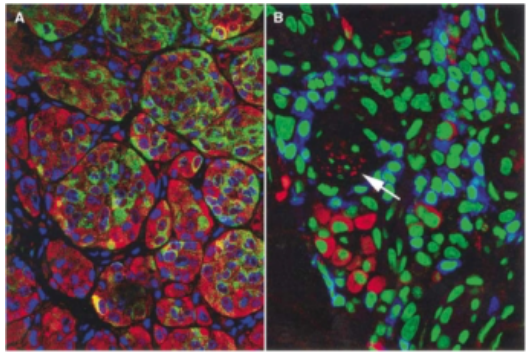
- Selective cytotoxicity
  - Tumor ICD
- Induction of immunity
- Favorable safety profile



Intratumoral cytokines: IL-2

Phase 2 study of 24 stage III and IV melanoma patients with IL-2 IT

- 245 lesions treated in 24 patients
- CR seen in 85% (n-209) of lesions and 62.5% of patients (n=15)
- PR seen in 6% (n=21) of lesions and 21% (n=5) of patients
- Toxicity limited to grade 1-2 events

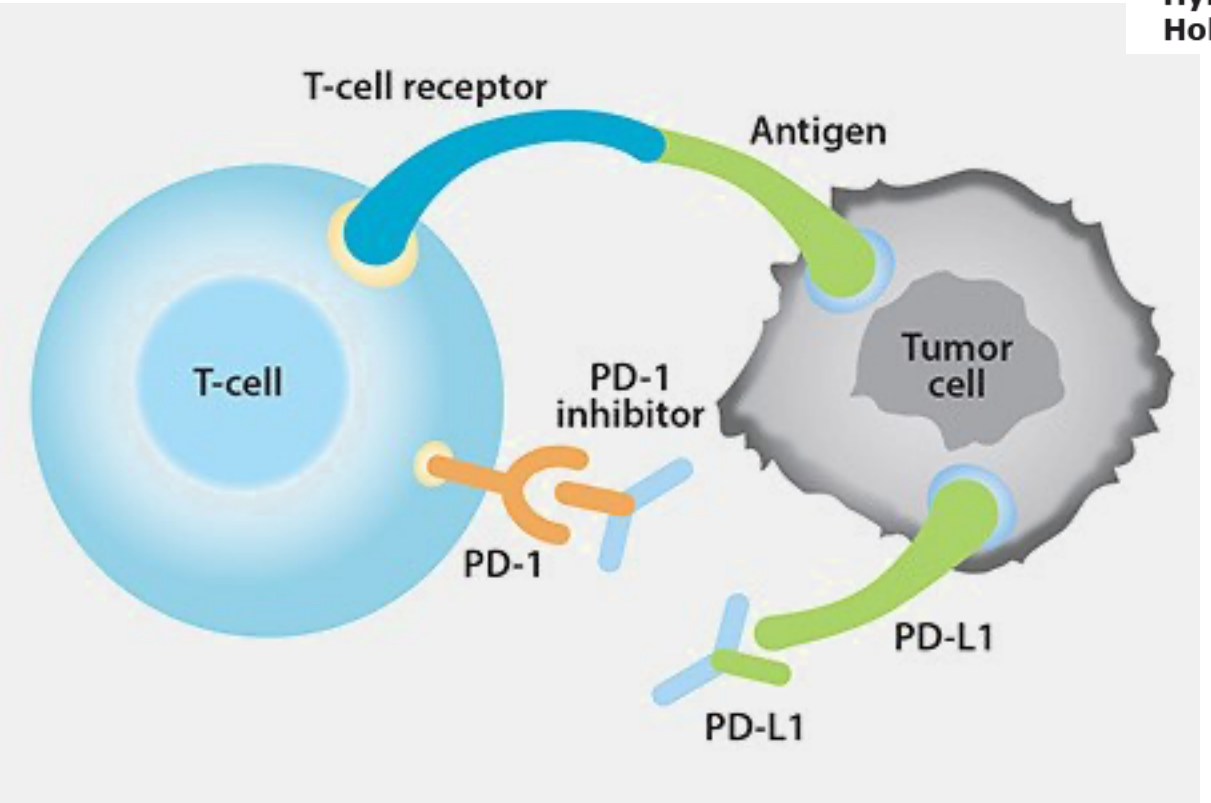


Meta-analysis of 49 studies of intra-lesional IL-2 for in-transit melanoma

- Six studies met criteria for analysis
- Overall, 2,182 lesions in 140 patients were treated
- CR occurred in 78% of lesions
- CR occurred in 50%
- Treatment well tolerated
  - Local pain and swelling
  - Mild flu-like syndrome
- Only three grade 3 adverse events
  - Rigors, Headache, Fever and Arthralgia

Radny et al. BR J Cancer 2003
Byers et al. J Surg Oncol 2014

Intratumoral immune checkpoint inhibitor mAbs



www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 39

Clinical Research Paper

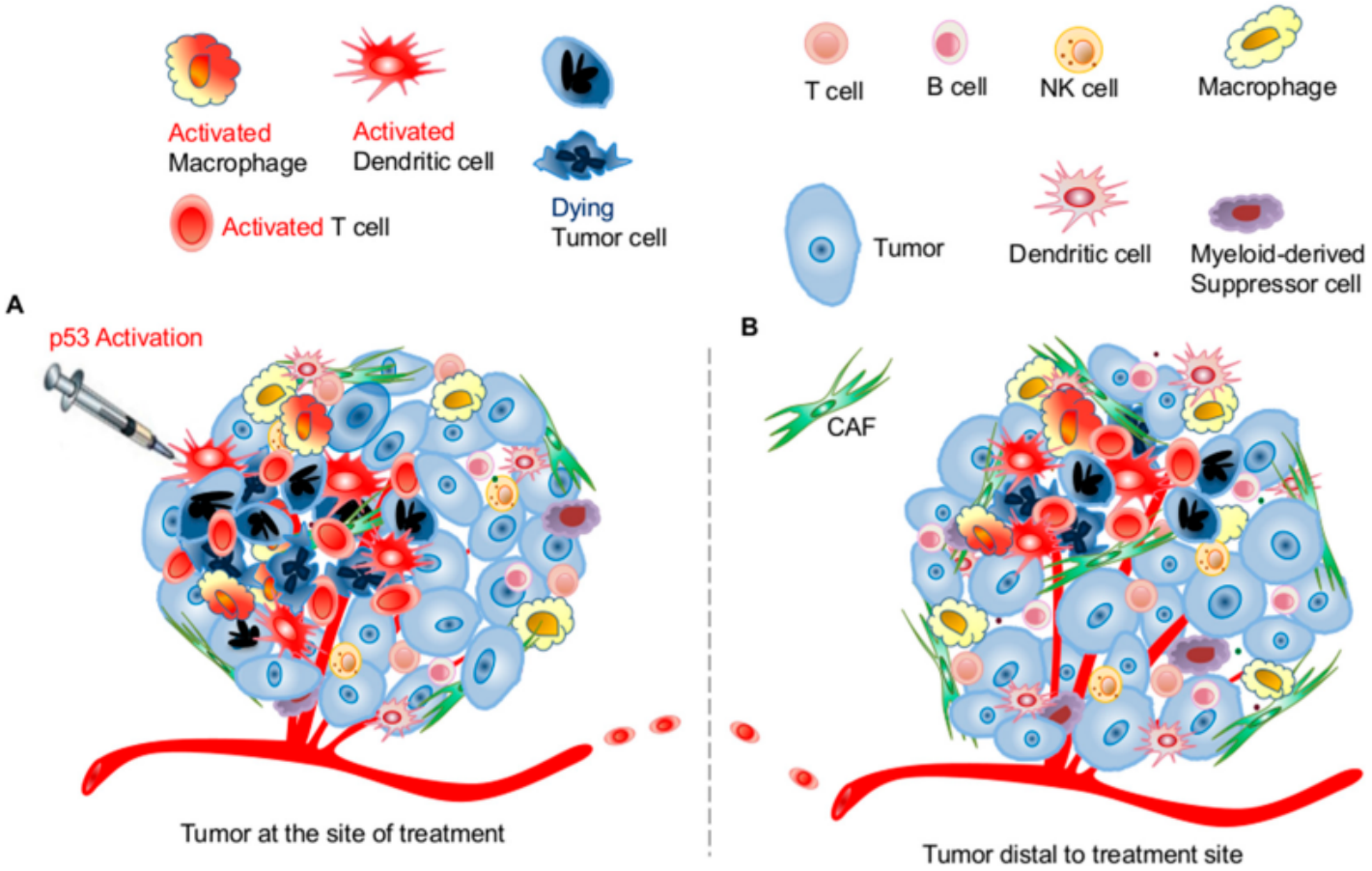
A phase I study of intratumoral ipilimumab and interleukin-2 in patients with advanced melanoma

Abhijit Ray[1,*], Matthew A. Williams[2,*], Stephanie M. Meek[2], Randy C. Bowen[3], Kenneth F. Grossmann[1], Robert H.I. Andtbacka[4], Tawnya L. Bowles[5], John R. Hyngstrom[4,5], Sancy A. Leachman[6], Douglas Grossman[1], Glen M. Bowen[1], Sheri L. Holmen[1], Matthew W. VanBrocklin[1], Gita Suneja[7] and Hung T. Khong[1]

- 12 patients; 3+3 design; 8 weeks of tx
- IL-2 at 3 MIU and dose escalation of ipilimumab (0.5 – 2 mg)
- No DLTs
- Grade 3 events of hyponatremia (1) and local ulceration (5)
- Local response 67%
- Abscopal response 89%
- ORR by irRC 40%

Courtesy Genekor
Ray et al. Oncotraget 2016

Intratumoral cell therapy (DC, T cells, etc.)

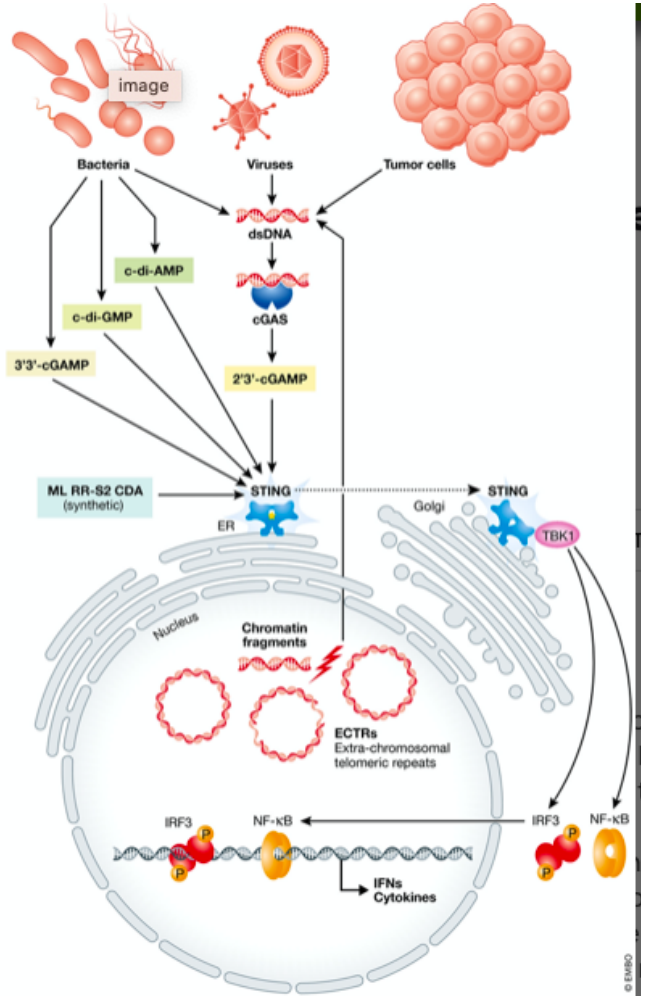


- Ex vivo modified cells
- In vivo modified cells

- Adoptive transfer and CART depend on recruitment to and function within the TME

Cui and Guo Int J Mol Sci 2016

Intratumoral STING immune agonists



- Stimulator of Interferon Genes
- Identified by expression cloning using IFN-beta reporter
- Allows foreign DNA sensing at the intra-cellular level
- Activates innate immunity
- Potent anti-viral activity
- ‘Senses’ tumor DNA
- Agonizing STING can promote anti-tumor activity

Khoo and Chen EMBO Rep 2018

Toll-like receptor agonists

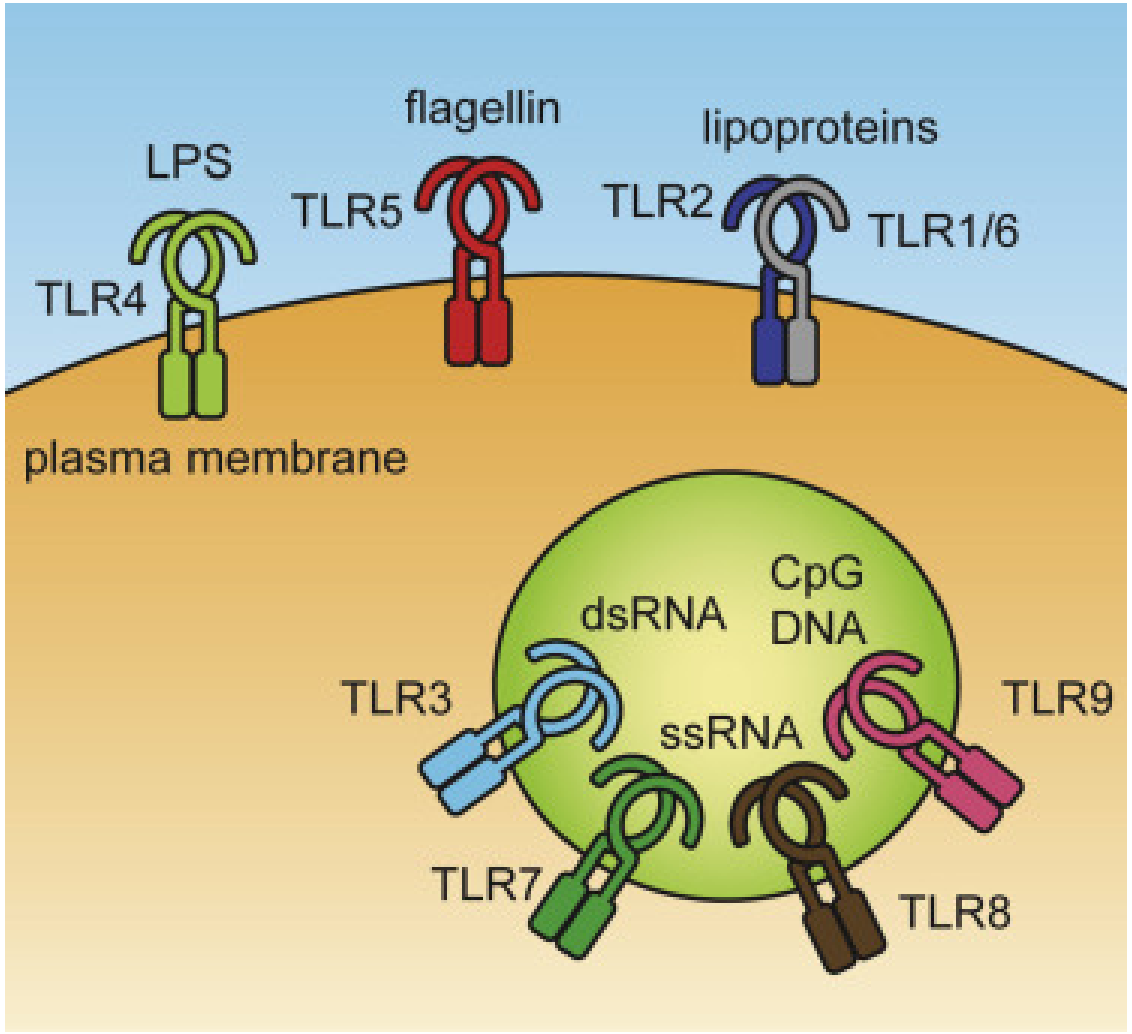
Danger is represented by:

These have molecular features that distinguish them from our own cells:

endotoxin
DS RNA
HMGB1
lipopeptides
CpG DNA
SS RNA
binds RAGE, LPS;
amplifies TLR sig
flagellin

Our immune systems have evolved to recognize them:

Obeid J, et al. *Semin Oncol.* 2015;42(4):549-561.



Ossenbrug et al. Cell Chem Biol 2017

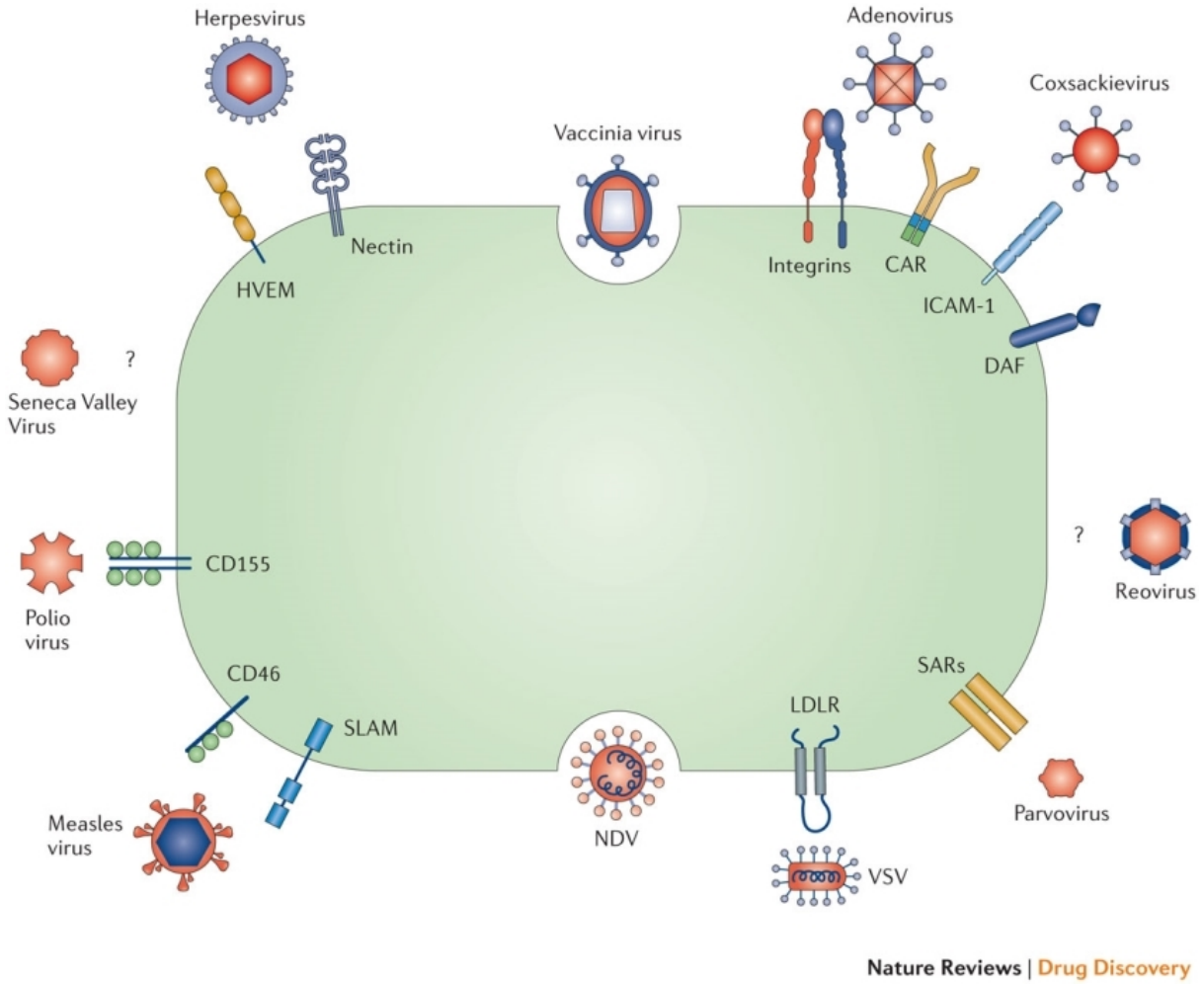
Intratumoral Immunotherapy

Pre-clinical Issues

Pre-clinical Issues

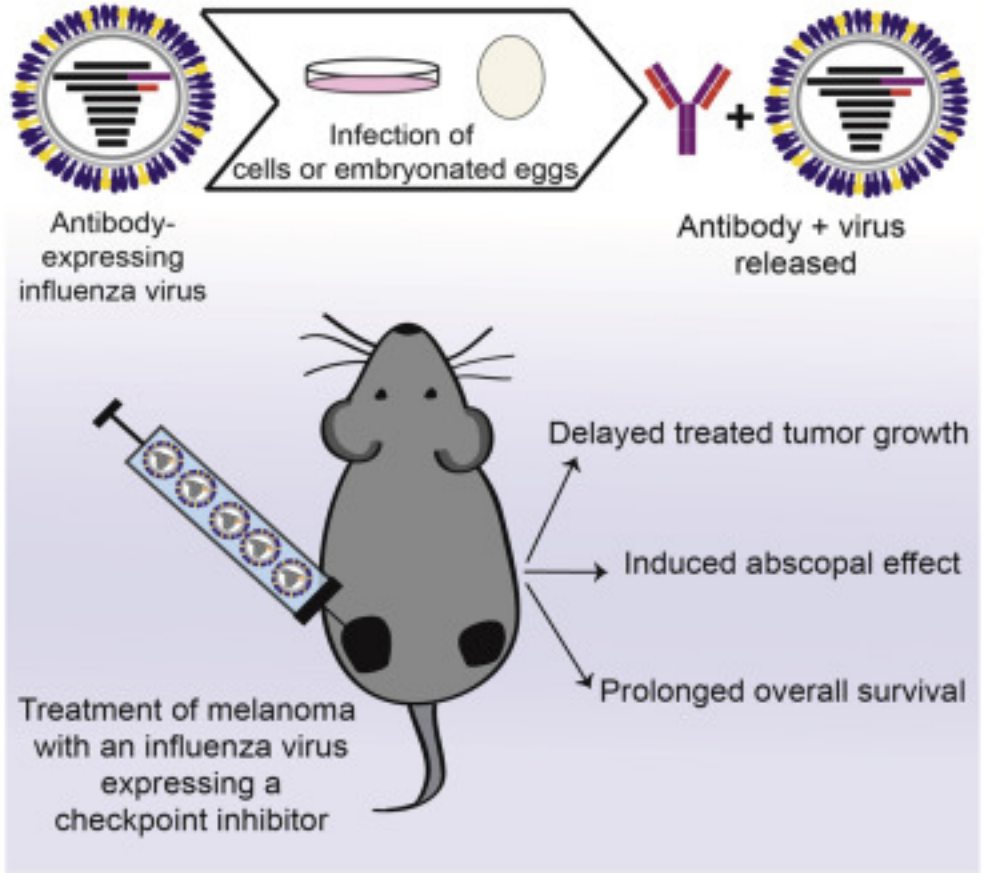
- Are tumor cells sensitive to drug entry?
- Are tumor cells killed? How?
- Biodistribution is important
  - Does drug remain in tumor (i.e. tumor cell restriction)?
  - Does drug leak to other sites (i.e. other cells in TME, distant tumors, normal tissue)?
- Need tumor model that incorporates injected and un-injected tumor (i.e., Is there an abscopal or anenestic effect?)
- Dose-response relationships should be defined
  - Anti-tumor vs. anti-viral immunity
- Dosing schedule and routes are important to validate

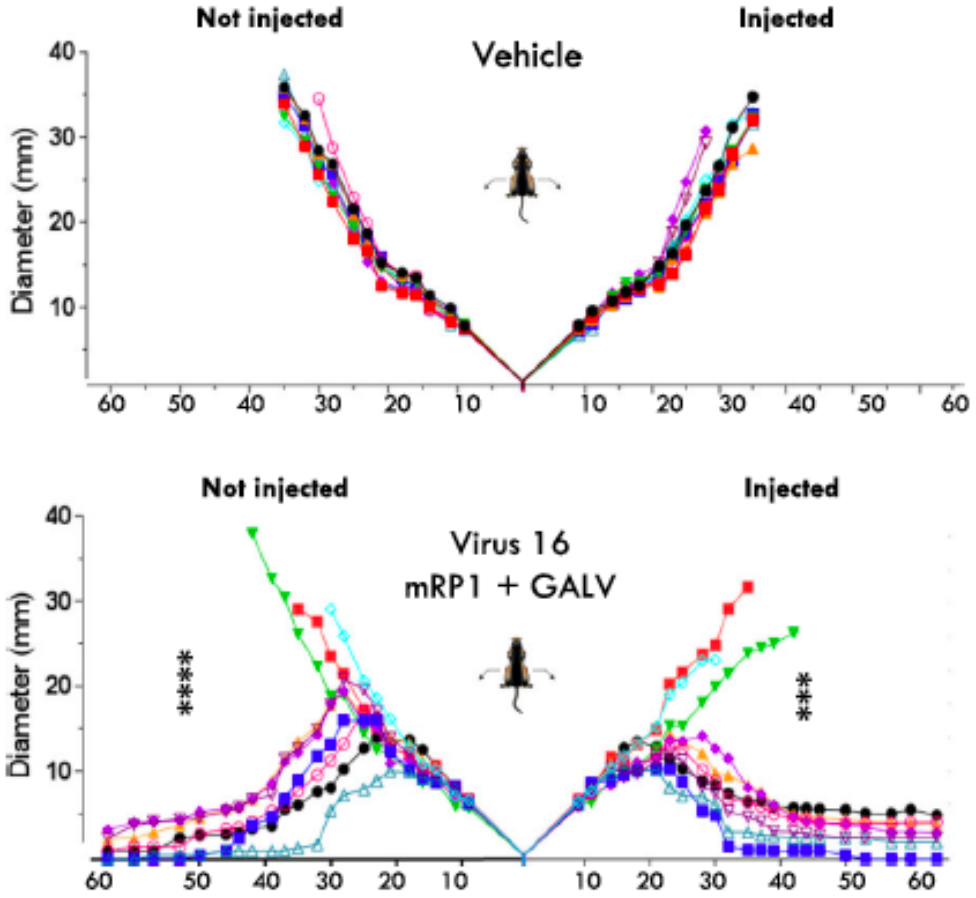
Oncolytic viruses utilize specific cell surface entry receptors



Kaufman, Kohlhapp, and Zloza Nat Rev Drug Discov. 2015 Sep;14(9):642-62

Intratumoral therapy should report injected and un-injected tumor responses

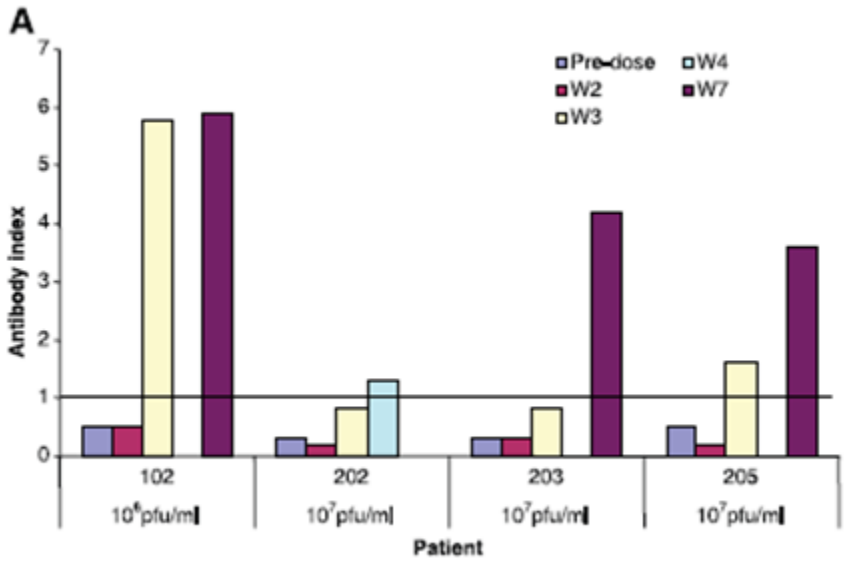




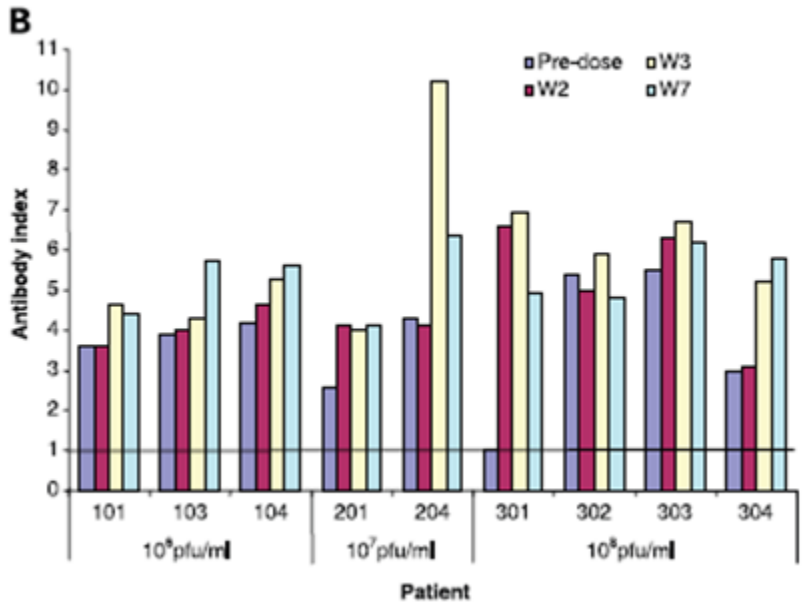
Hamilton et al. Cell 2018
Thomas et al. JITC 2019

Consideration of anti-viral immune response

Anti-HSV-1 Ab titers



HSV-1 Seronegative at Baseline



HSV-1 Seropositive at Baseline

Hu et al. Clin Cancer Res 2006

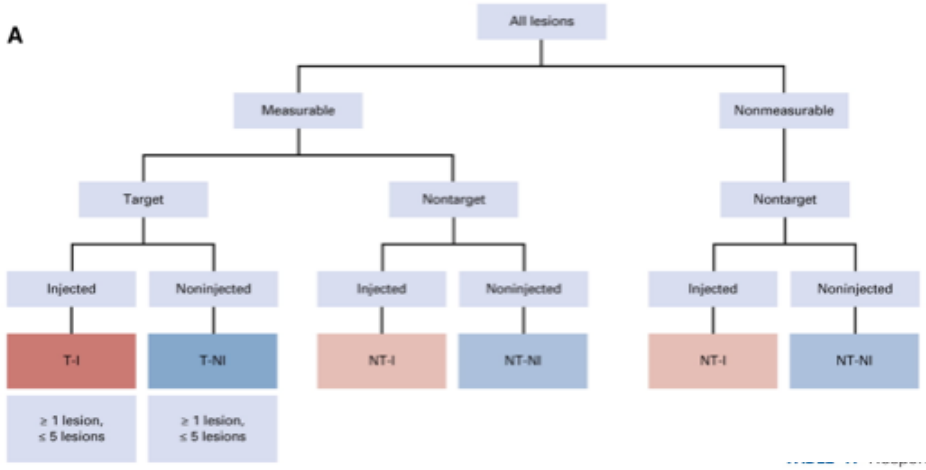
Intratumoral Immunotherapy

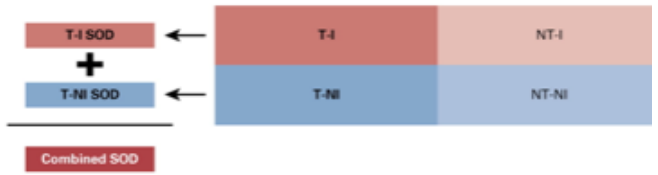
Clinical and Logistical Issues

Clinical Issues

- Subject eligibility
  - Tumor size
  - Tumor location (e.g., access)
- Drug delivery
  - Dose vs. volume
  - Schedule
  - Intra-tumoral vs. intra-venous
  - Which lesions to inject or treat?
- Endpoints
  - Injected (treated) lesions
  - Un-injected (un-treated) lesions [abscopal or anenestic responses]
  - Biomarkers (local vs. distant or systemic)

Intratumoral RECIST (itRECIST) for local immunotherapy





Response	Definition
T-I lesions	
CR	All nonnodal lesions gone, nodal lesions < 10 mm
PR	≥ 30% decrease in SOD from last imaging assessment
PD	≥ 20% increase in SOD from last imaging assessment (≥ 5 mm absolute)
SD	Not enough growth for PD
	Not enough shrinkage for PR
NE	≥ 1 lesion cannot be measured
T-NI lesions	
CR	All nonnodal lesions gone, nodal lesions < 10 mm
PR	≥ 30% decrease in SOD from baseline
PD	≥ 20% increase in SOD from nadir (≥ 5 mm absolute)
SD	Not enough growth for PD
	Not enough shrinkage for PR
NE	≥1 lesion cannot be measured or has been injected

Abbreviations: CR, complete response; NE, nonevaluable; PD, progressive disease; PR, partial response; SD, stable disease; SOD, sum of diameters; T-I, target injected; T-NI, target noninjected.

Goldmacher et al. JCO 2020

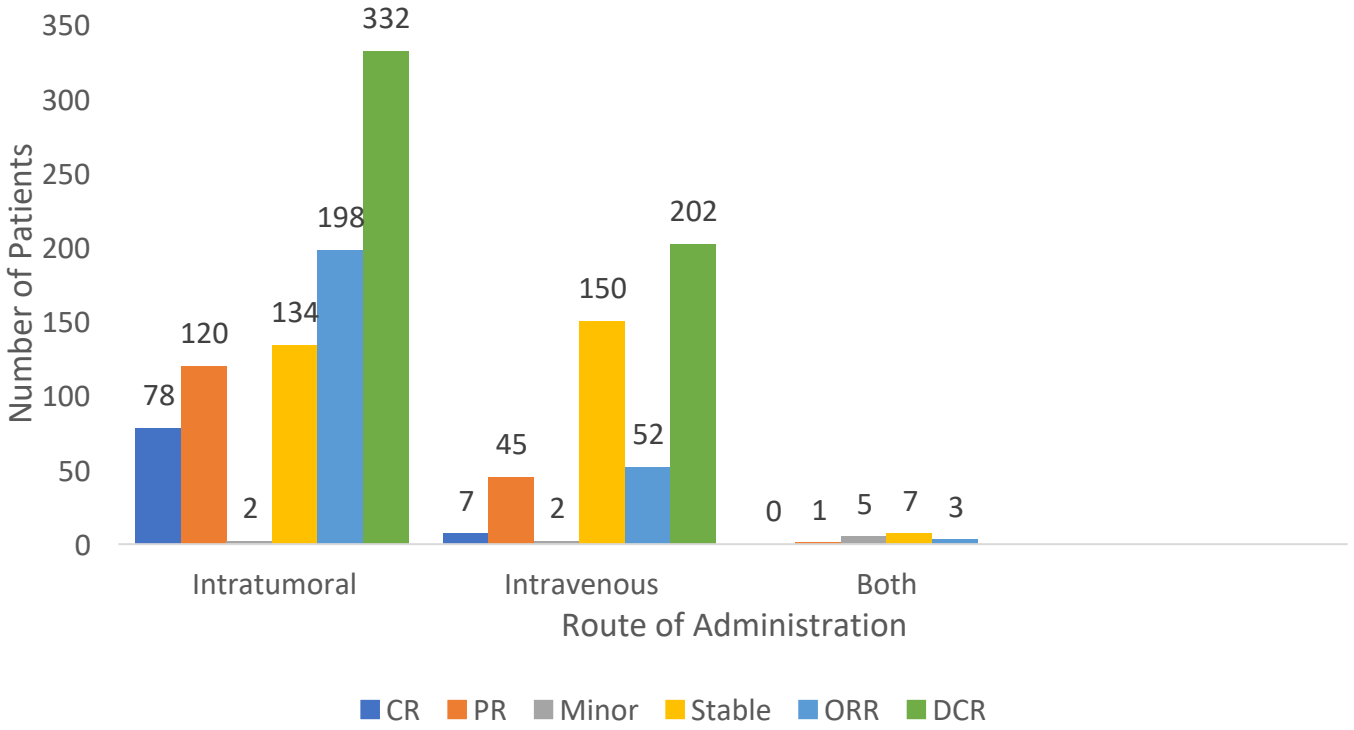
- Consider injected and un-injected lesions
- 1 vs 2 dimensions (RECIST vs. WHO)
- Imaging of cutaneous lesions imperfect
- Photography helpful but time consuming
- “Pseudo-progression” may be common
- Complete regression may be hard to define
- Role for biopsy confirmation?
- irRECIST has not been validated

➢ Modified RECIST
  ➢ Allow treatment post progression
  ➢ Use standard RECIST

Logistical issues associated with intra-tumoral immunotherapy

- Drug delivery
- Access to visceral sites
  - Image-guided delivery is possible
  - Some sites challenging (e.g., brain, bone, liver dome, etc.)
- Biosafety issues
- Leaking from the tumor site
- Endpoint assessment
  - Need to document injected sites and non-injected sites
  - Abscopal (anenestic) responses may utilize different MOA, kinetics

Intravenous delivery of IT agents



- Easier route to administer
- Potentially targets all metastatic lesions
- To date, appears safe

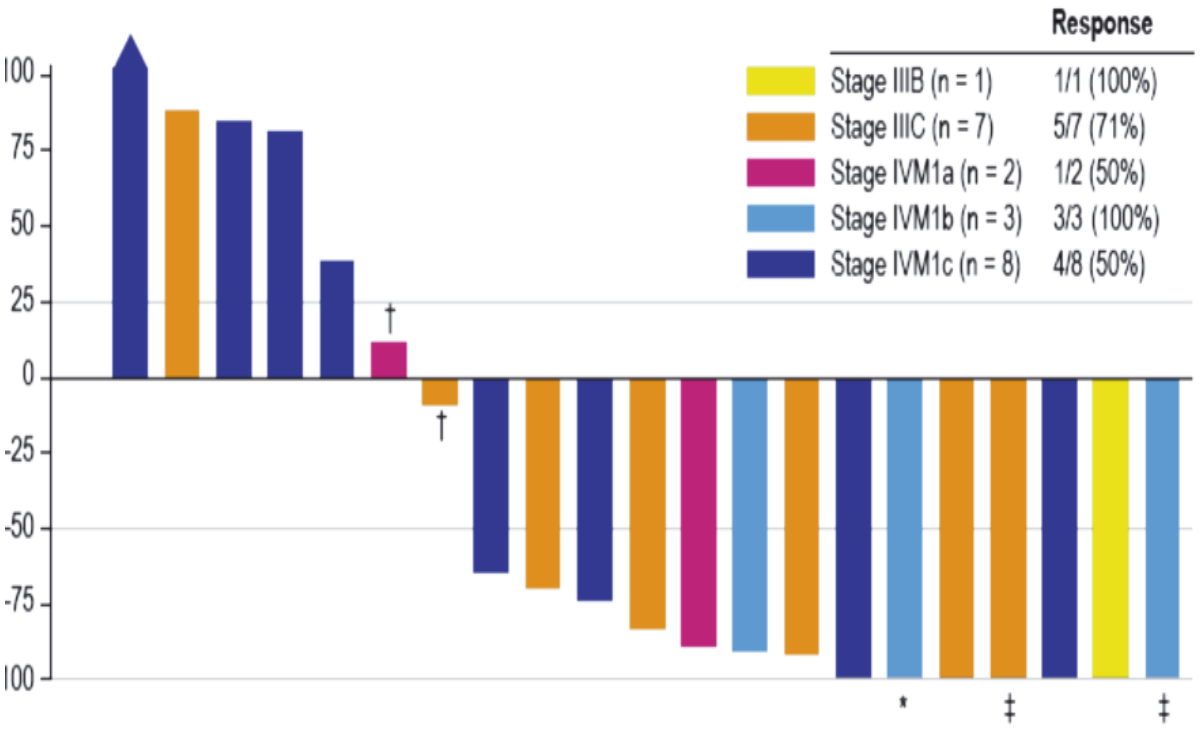
- But,
- Limited biodistribution a challenge
  - Immune clearance (i.e., Abs, complement)
  - Protein sequestration
- To date, limited efficacy reported
- Few studies report viable drug at tumor site

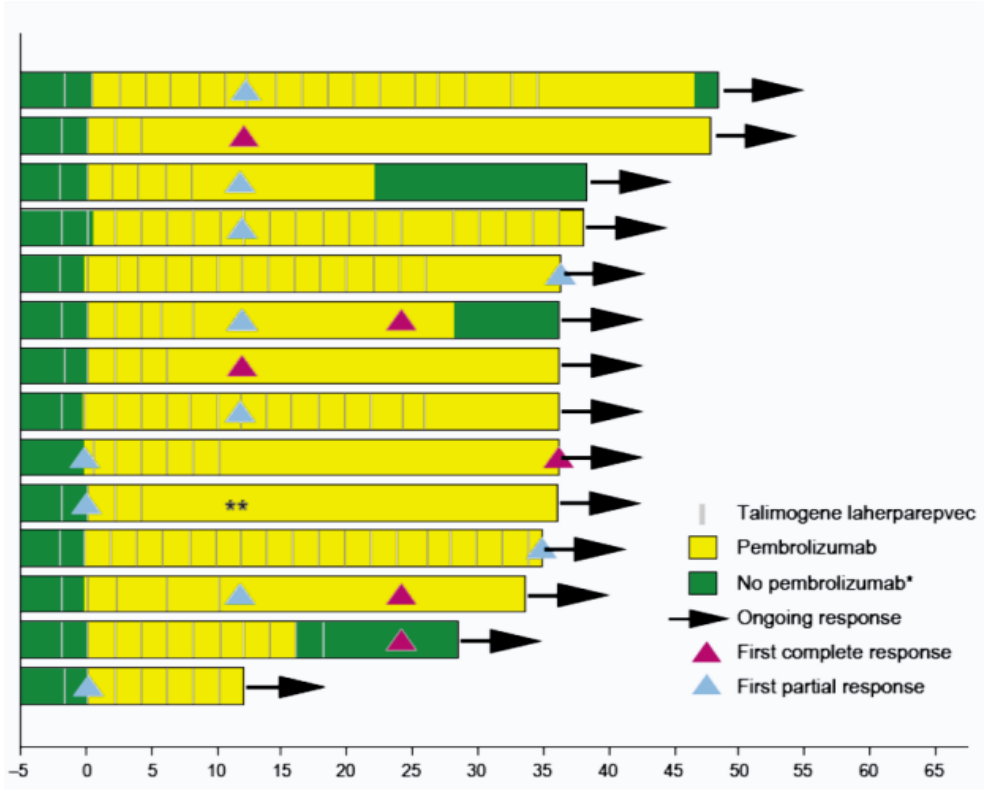
Macedo, et al. JITC 2020

Intratumoral Immunotherapy

Integrating Into Combination Therapy

Phase 1 clinical trial of T-VEC and pembrolizumab in melanoma

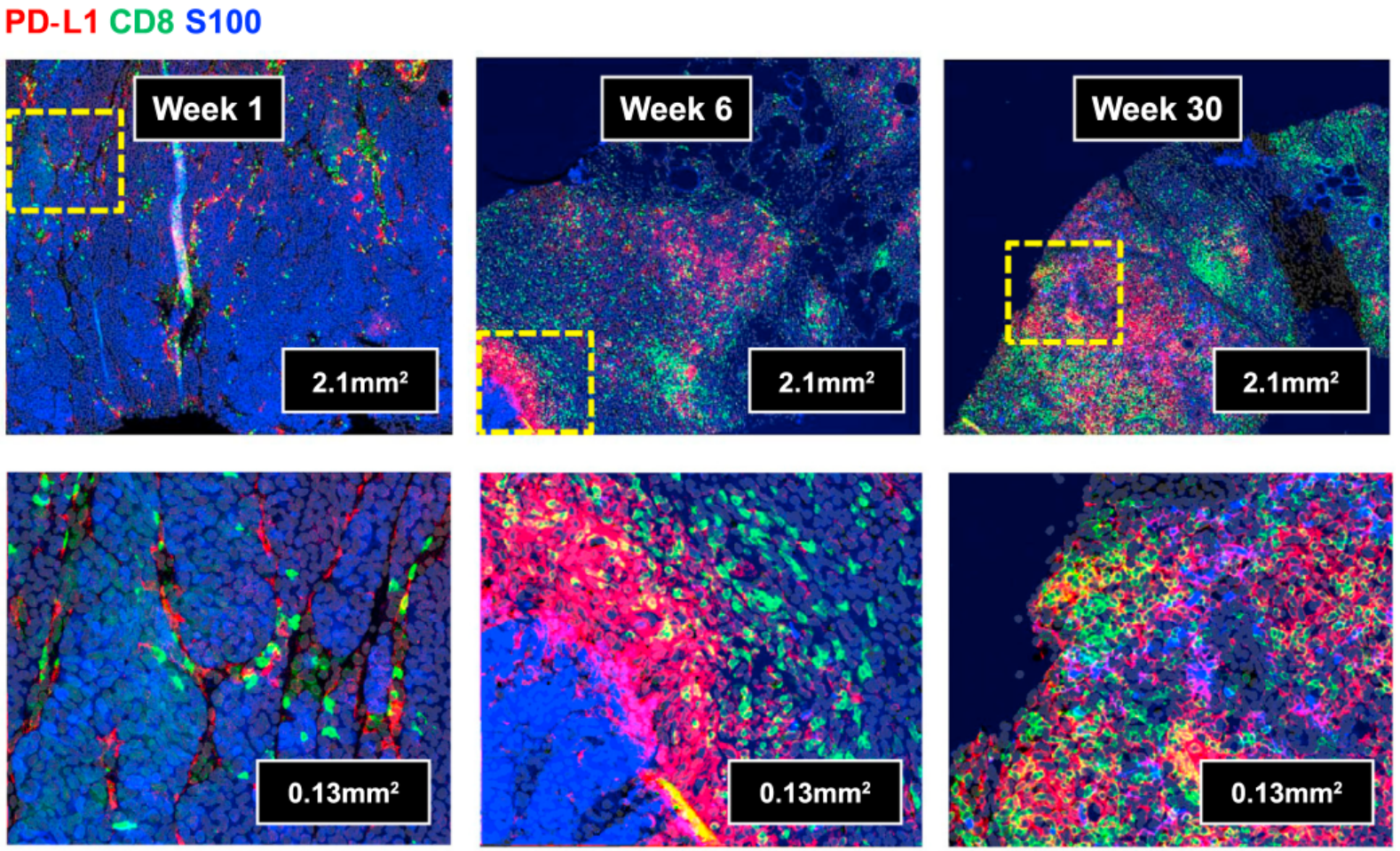




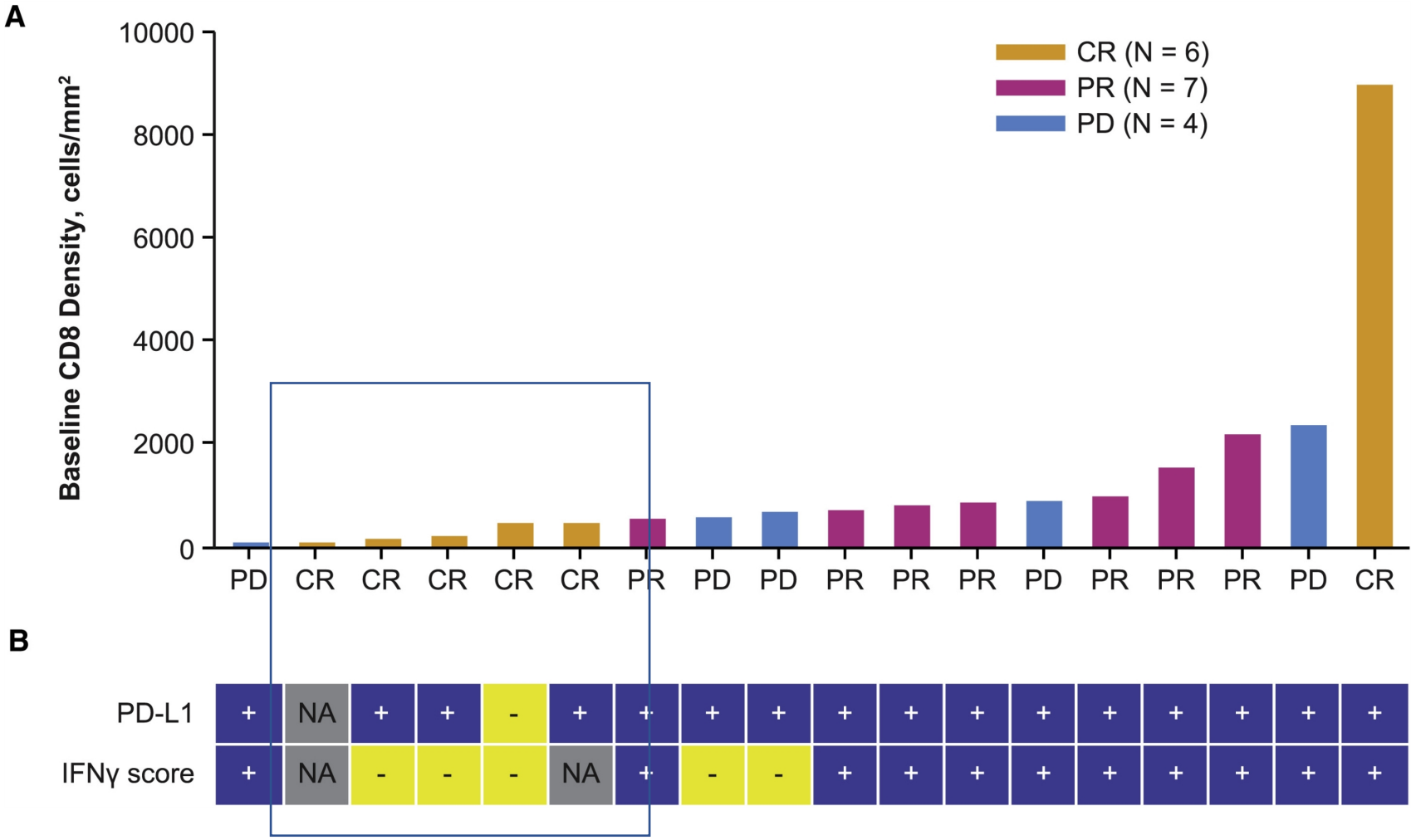
Without added toxicity

Ribas et al. Cell 2017

T-VEC induces CD8+ T cell recruitment and PD-L1 expression in the TME



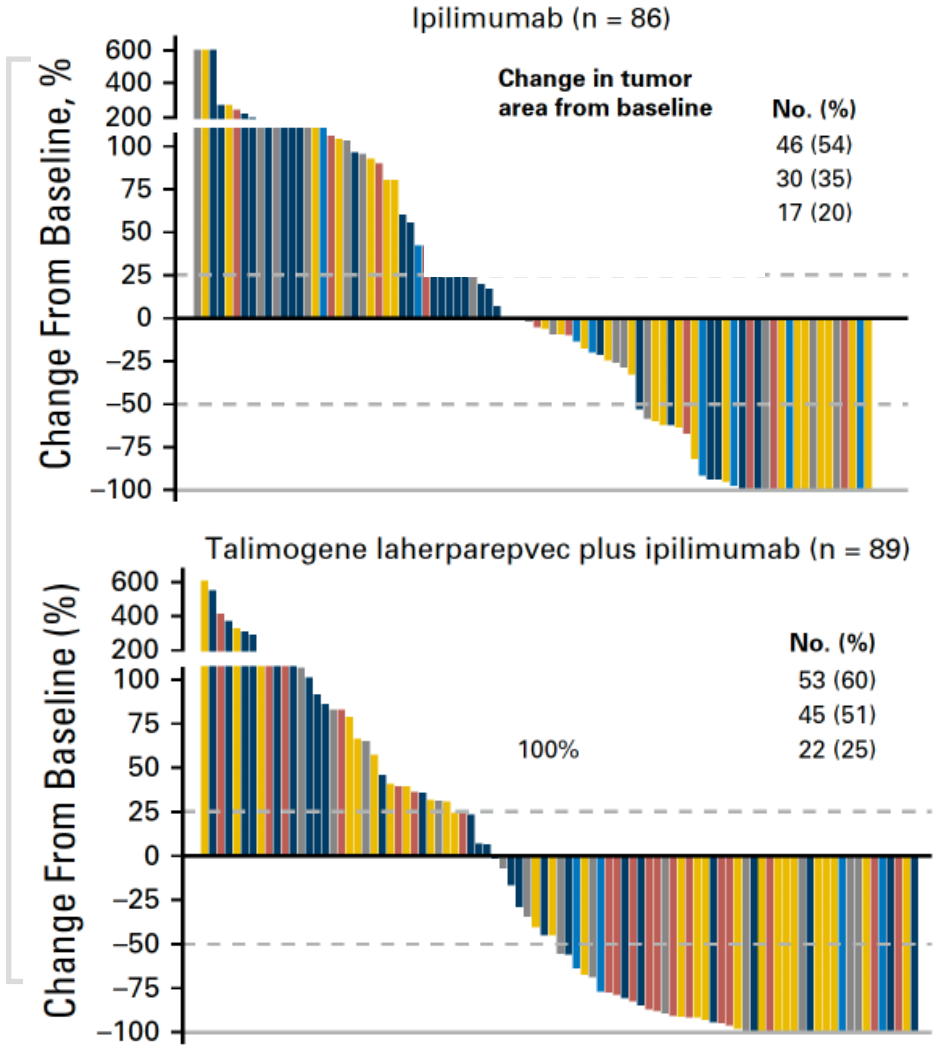
T-VEC + pembrolizumab induces CR in immunologically deserted tumors



Ribas et al. Cell 2017

Randomized Phase 2 Clinical Trial: T-VEC + ipilimumab improves ORR

- T-VEC + ipilimumab vs. ipilimumab alone Stage IIIb-IVM1c melanoma
- Response rates (N=198) more than doubled with T-VEC + ipilimumab vs. ipilimumab alone (38% vs. 18%)
- For visceral lesions (none injected), the response rate was 35% for T-VEC +ipilimumab vs. 14% for ipilimumab alone
- No additional toxicity as compared to ipilumumab alone



Chesney et al JCO, 2017

Outstanding Issues with IT therapy

- How should eligibility be modified from standard clinical studies?
- Regulatory requirements for biodistribution are evolving
- Should all tumor be injected?
- Can IT agents be delivered by intravenous route?
- What are appropriate clinical endpoints?
  - Monitoring of injected vs. un-injected lesions
- What is the optimal schedule for treatment (including when to stop), especially in combination with other agents?
- How should component contributions be confirmed?
  - Clinical vs. biomarker validation
- How long should contact transmission be monitored?
- Is neoadjuvant treatment better?

Conclusions

- Intratumoral immunotherapy is defined as local delivery of agents that induce innate/adaptive anti-tumor immune responses
- There are many types of intratumoral immunotherapy in clinical development
  - Physical approaches
  - Drug-based approaches
- Intratumoral immunotherapy pre-clinical considerations
  - Validate cell entry receptors, extent and type of cell lysis, local and distant anti-tumor activity in immune competent murine systems, immunogenicity
- Intratumoral immunotherapy clinical and logistical considerations
  - Must consider dosing, schedule, volume, biodistribution, anti-viral responses, eligibility and endpoint responses
- Intratumoral immunotherapy as part of a rational combination approach
  - Neoadjuvant, IO combinations, non-IO combinations