Intratumoral Immunotherapy

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Disclosures

- I am an employee of Ankyra Therapeutics
- I served on advisory board for Castle Biosciences

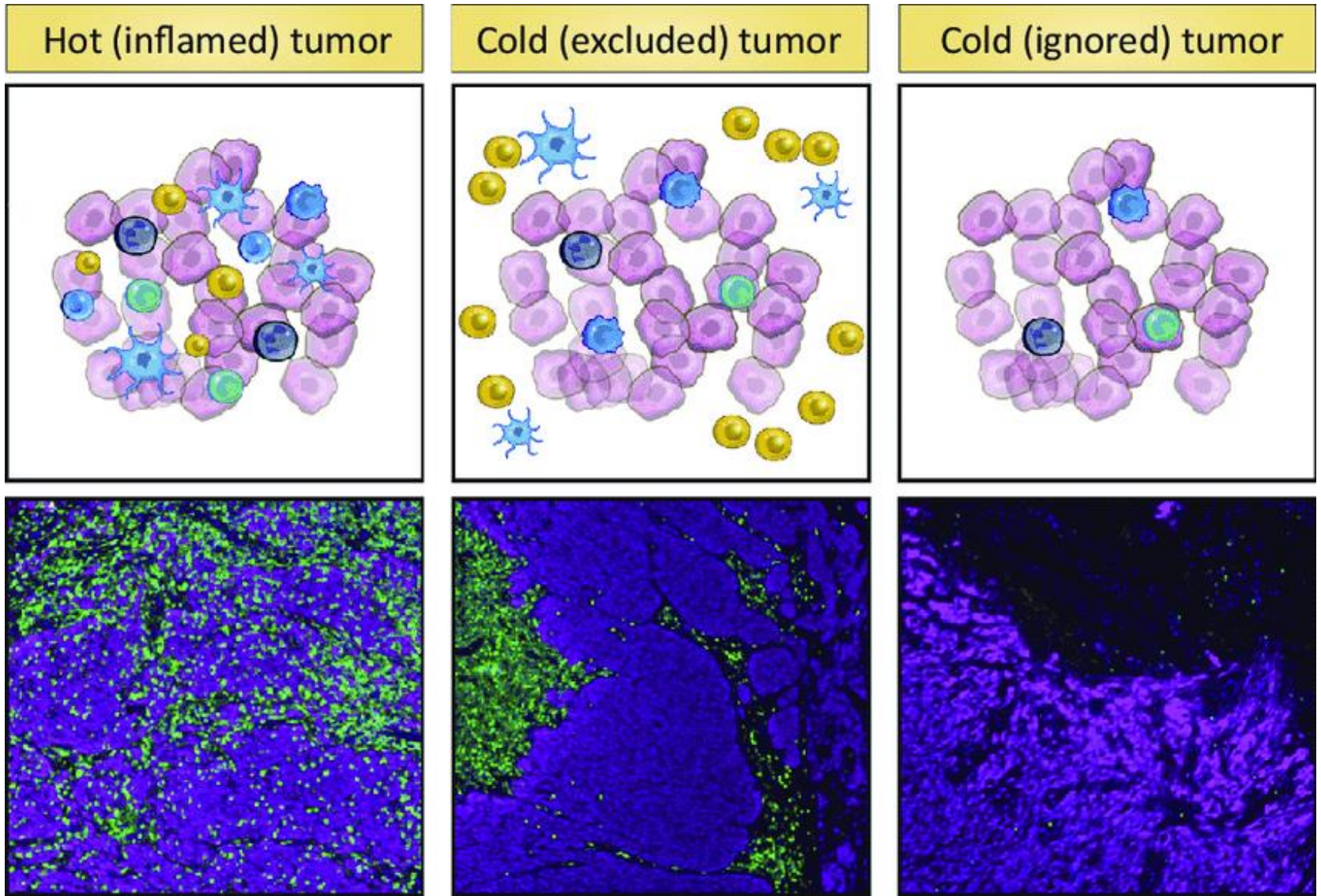
Intratumoral Immunotherapy

Definitions and Rationale

What is intra-tumoral Immunotherapy?

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

Hot vs. cold tumor microenvironment



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

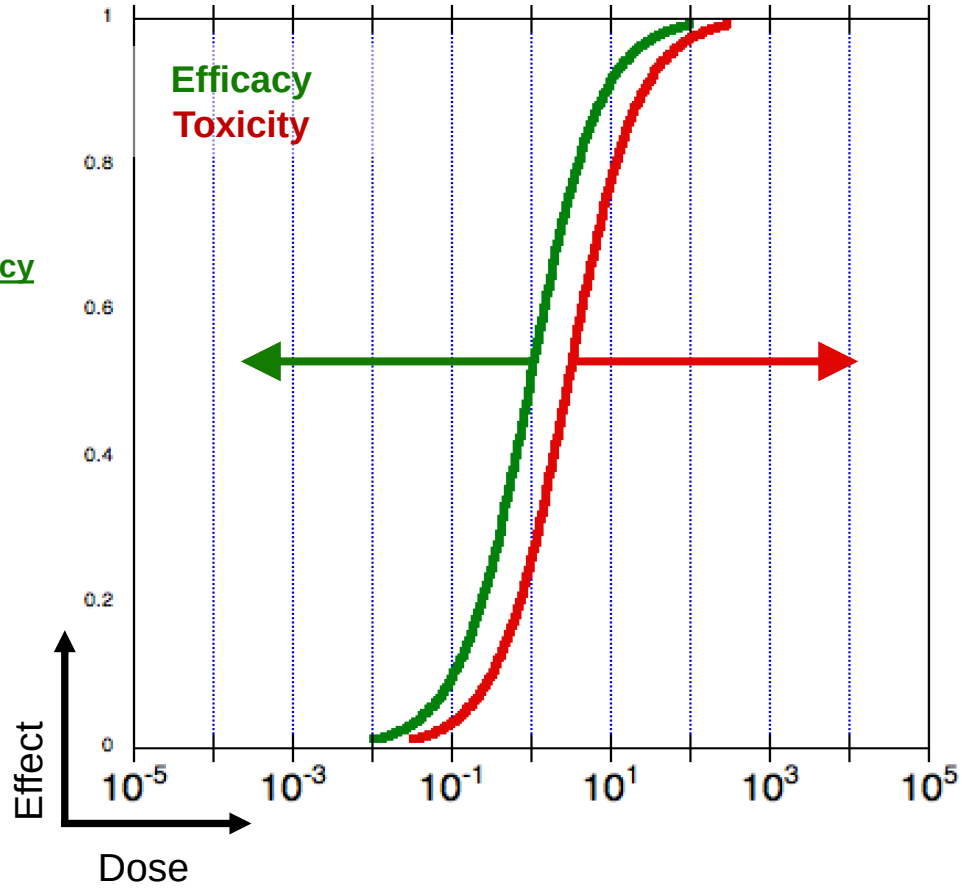
van der Woude et al. Trends Cancer 2017

IO agonists are limited by poor therapeutic windows

- Limited clinical success of systemically administered cytokines and antibody agonists
- On-target, off-tumor toxicity restricts dosing
- Transport barriers and immunosuppressive microenvironment of solid tumors limit efficacy

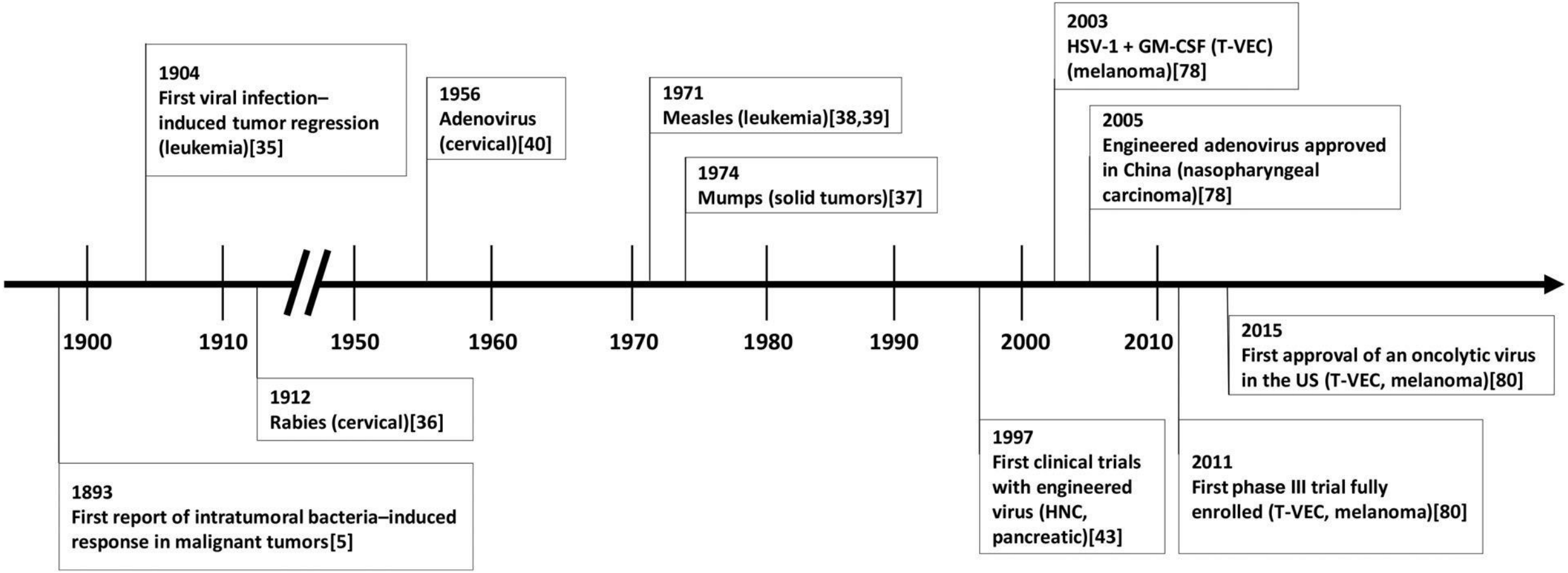
Intratumoral administration has potential to greatly expand therapeutic window by increasing relative tumor vs systemic exposure

Enhance Efficacy
Increase tumor exposure



Reduce Toxicity
Decrease systemic exposure

History of Intra-tumoral Therapy of Cancer

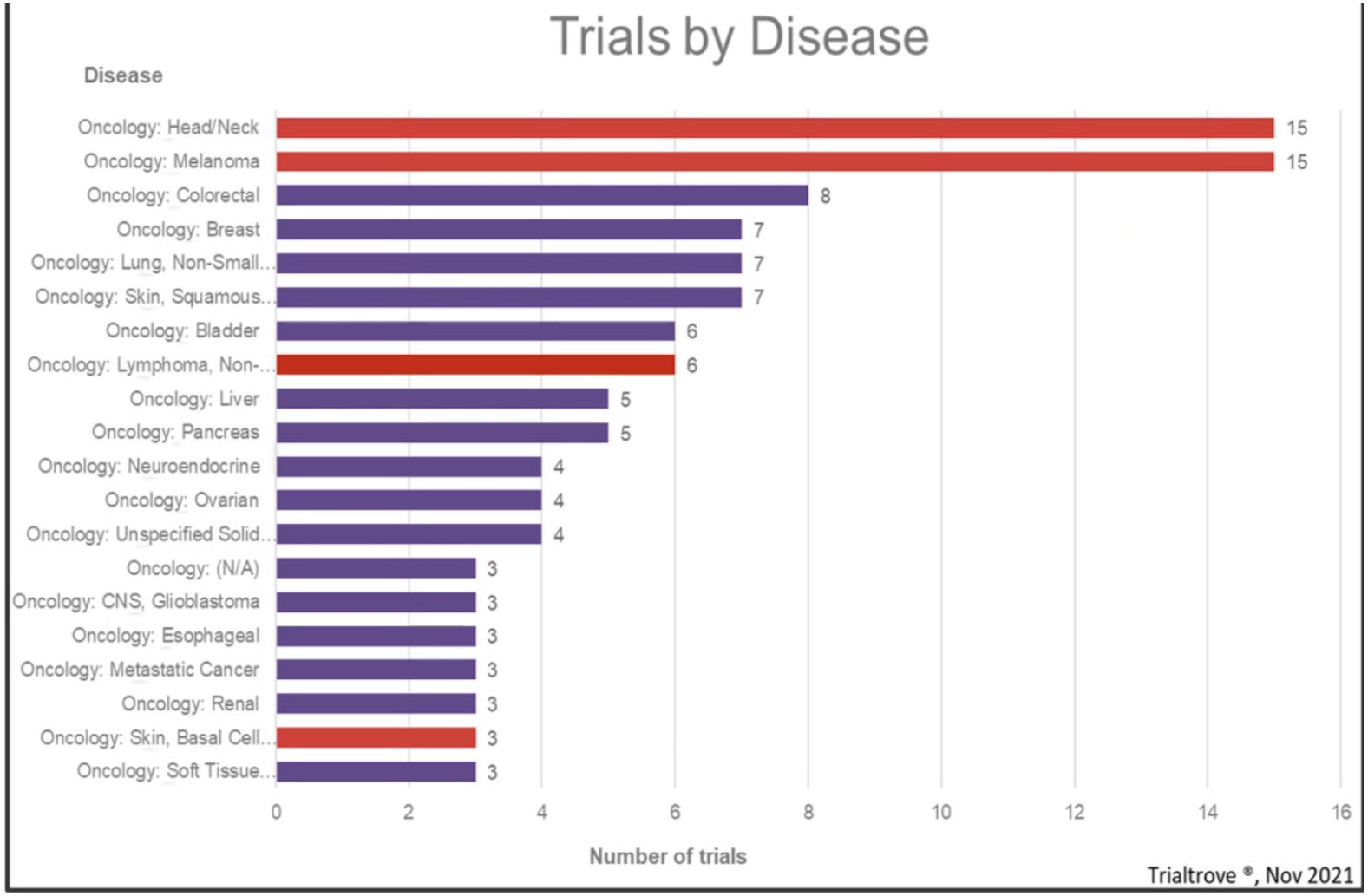


Hamid and Puzanov The Oncologist 2019

Global Approved Oncolytic Viruses for Cancer

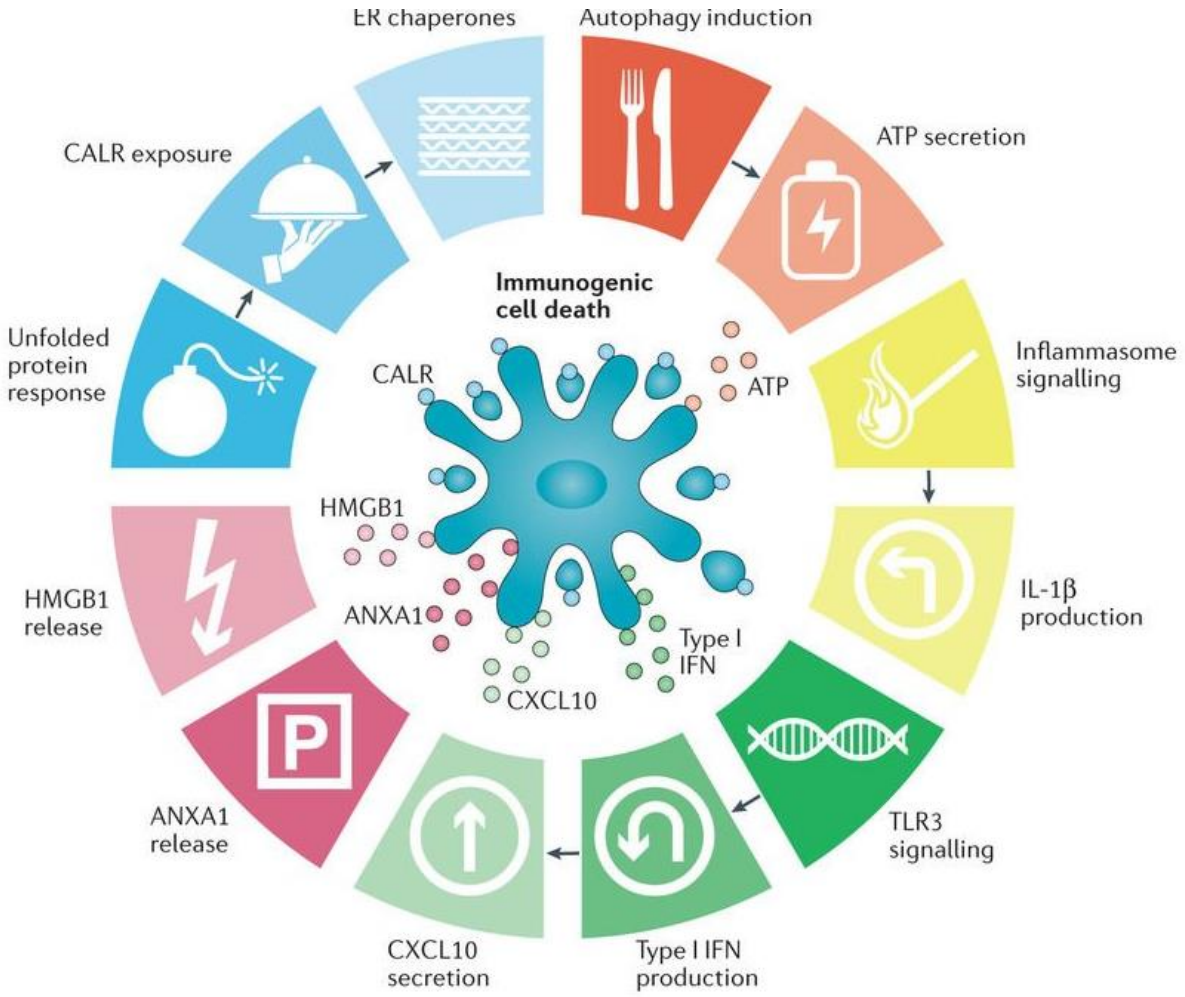
Name	Virus	Indication	Country	Year Approved
H101 (Oncorine®)	Adenovirus	Nasopharyngeal carcinoma (with chemotherapy)	Peoples Republic of China	2005
Talimogene laherparepvec (T-VEC; Imlygic®)	HSV-1-GM-CSF	Melanoma	United States Europe Israel Australia	2015
ECHO-7 (Rigvir®)	Echovirus (picornavirus family)	Melanoma	Latvia Georgia Armenia	2019
Teserpaturev	HSV-1	Malignant Glioma	Japan	2021

Number of Intratumoral Clinical Trials in Oncology as of 11/30/2021



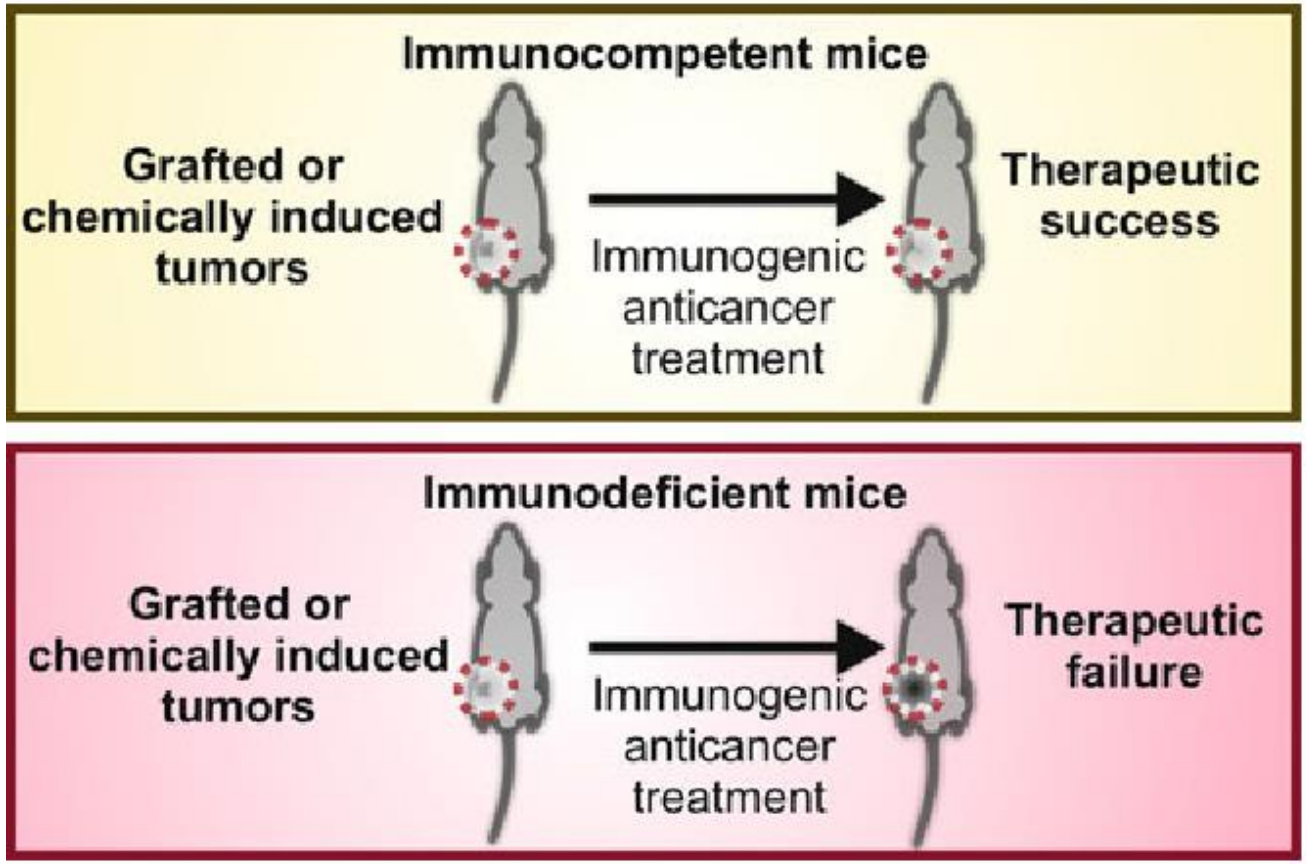
- 82 active IT clinical trials
  - 65 active
  - 17 planned
- 42 trials in the U.S.
- 45 trials in Phase I or I/II

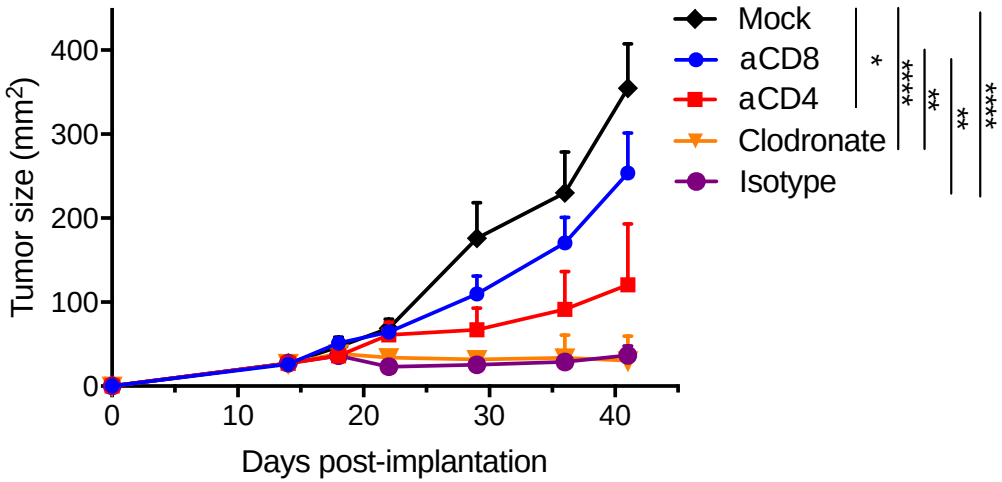
Intralesional approaches Induce immunogenic cell death



Galluzzi et al. Nature Immunol. 2017

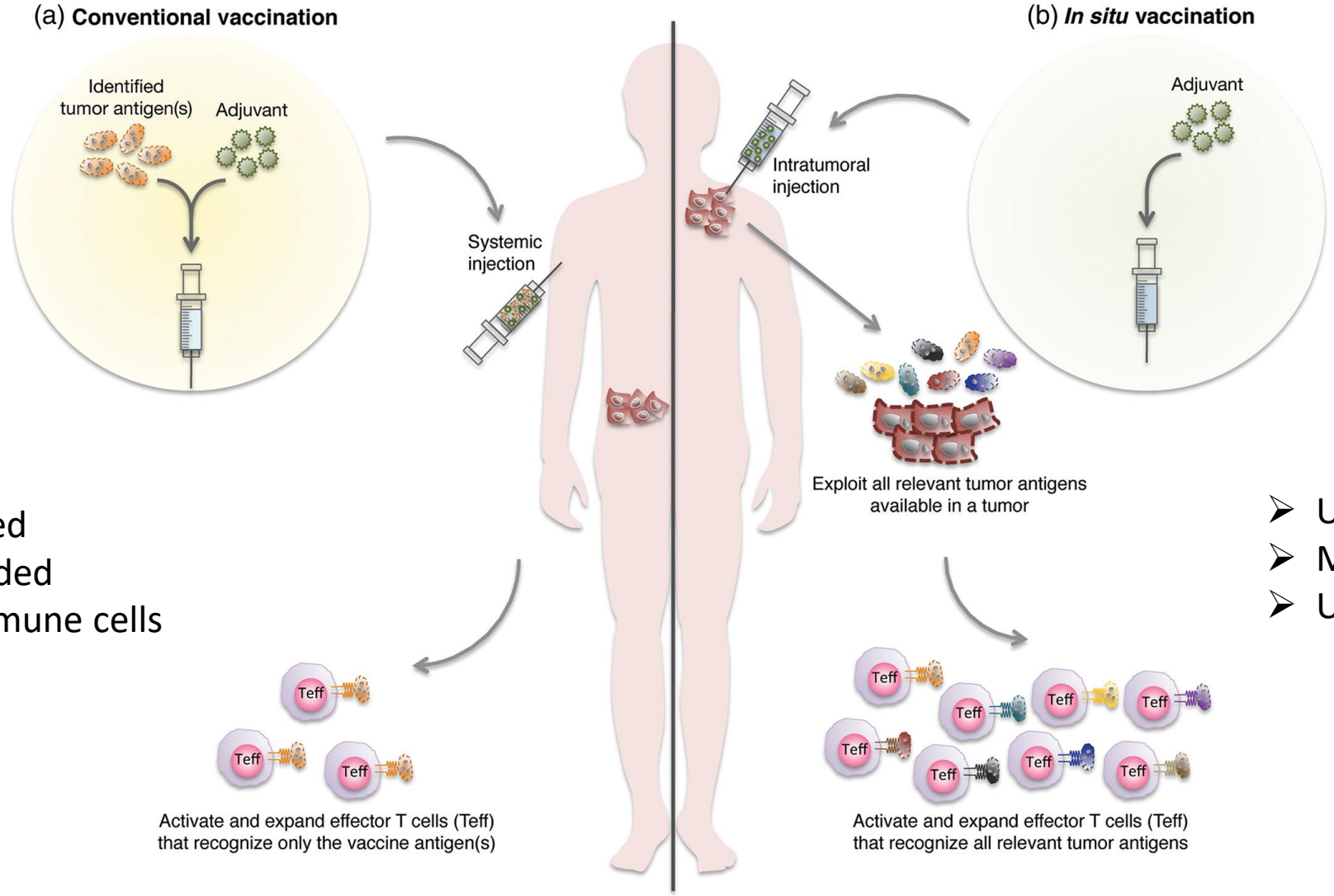
Contemporary definition of ICD





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

Intratumoral immunotherapy may have an *in situ* vaccination effect



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

- Uses native antigens
- Must access tumor
- Uses local immune system

Sheen and Fiering WIREs 2018

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
- Less expensive
- May preclude or delay need for more toxic systemic agents

Intratumoral Immunotherapy

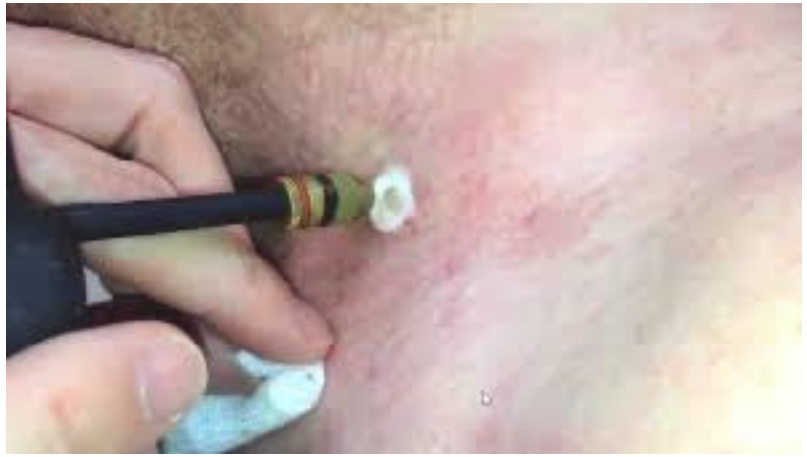
Types of Intratumoral Therapy

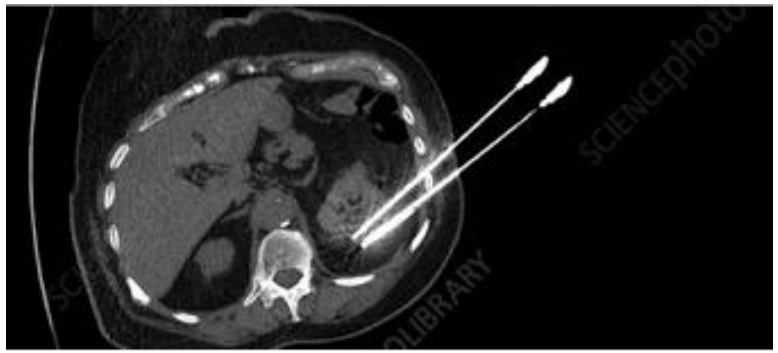
Types of Intra-tumoral therapy

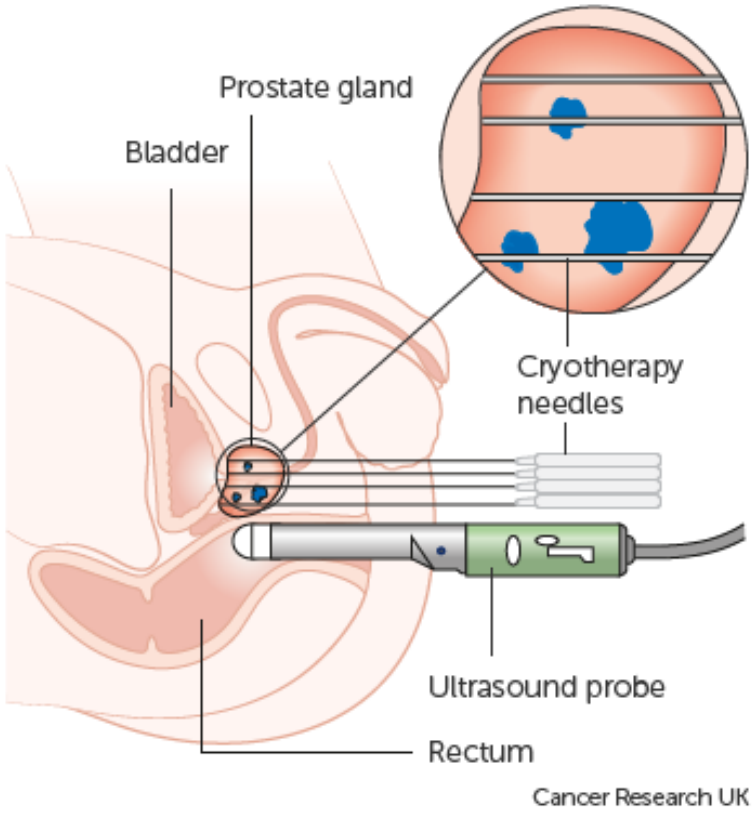
- Physical (Ablative) therapies
- Drug-related therapies
- Intravenous delivery with local activity
- Combination therapy

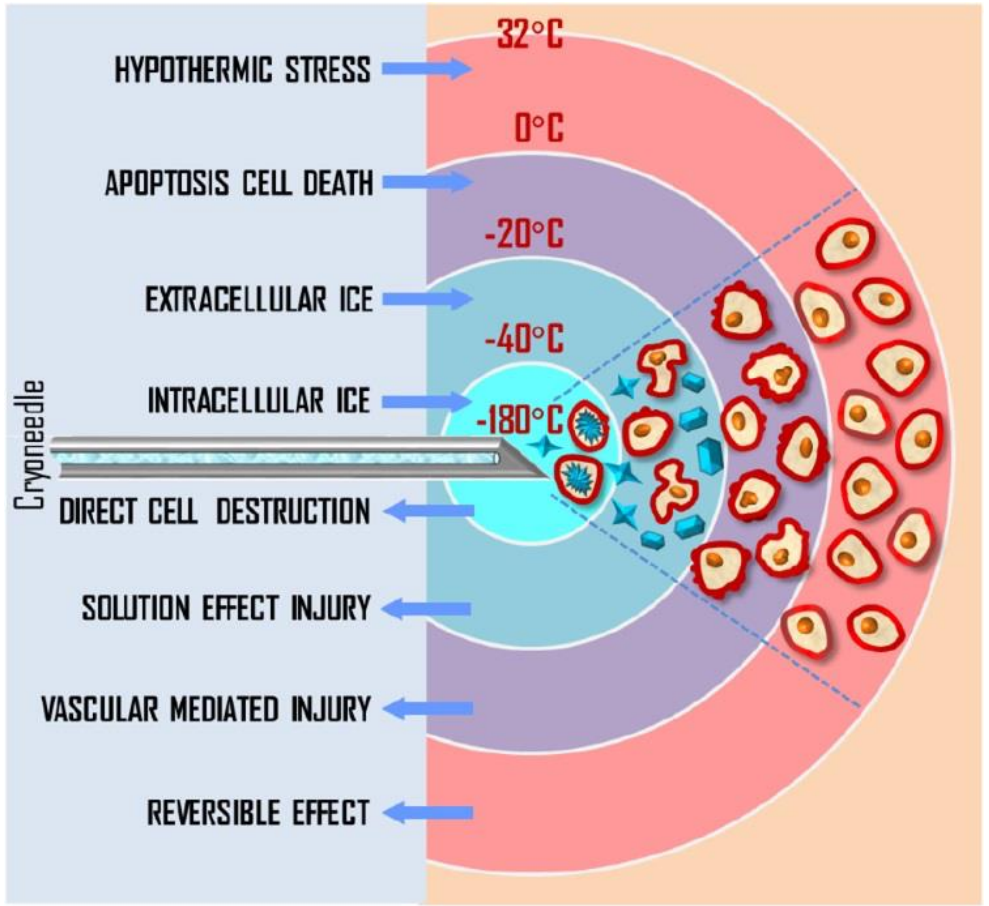
Physical Intratumoral Therapy

Cryotherapy





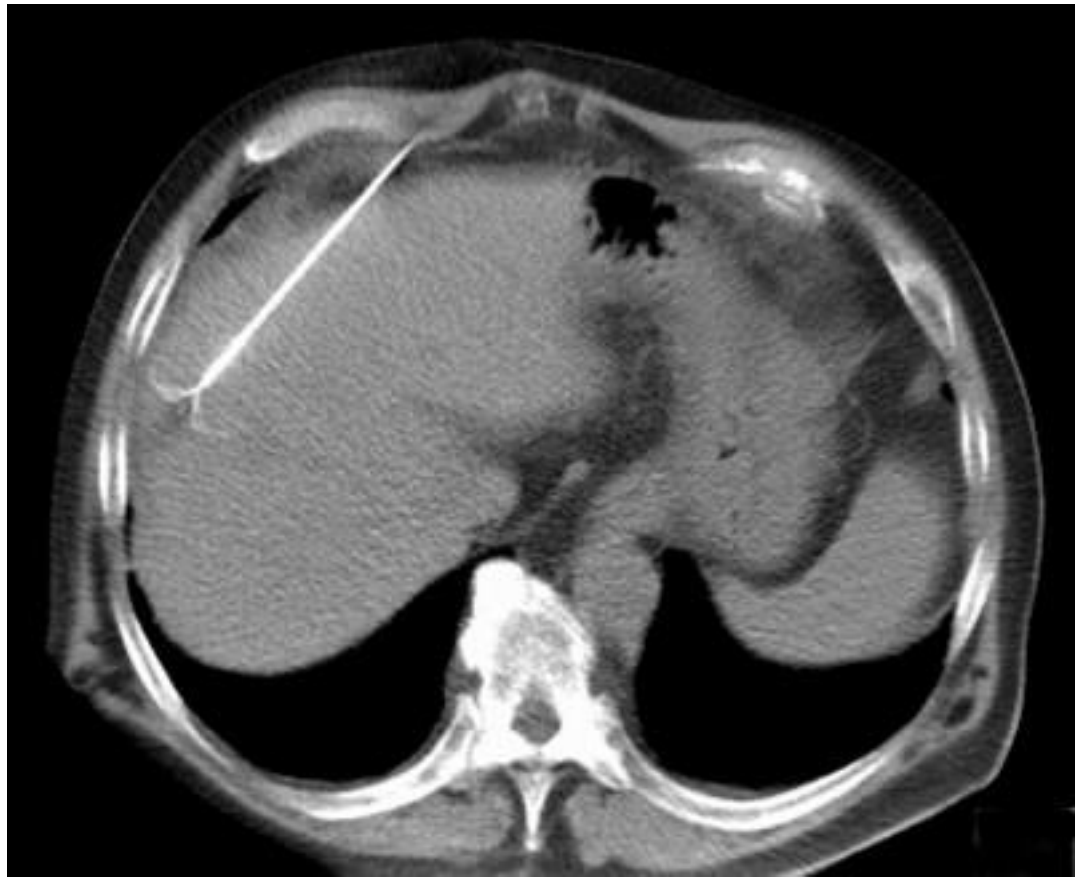


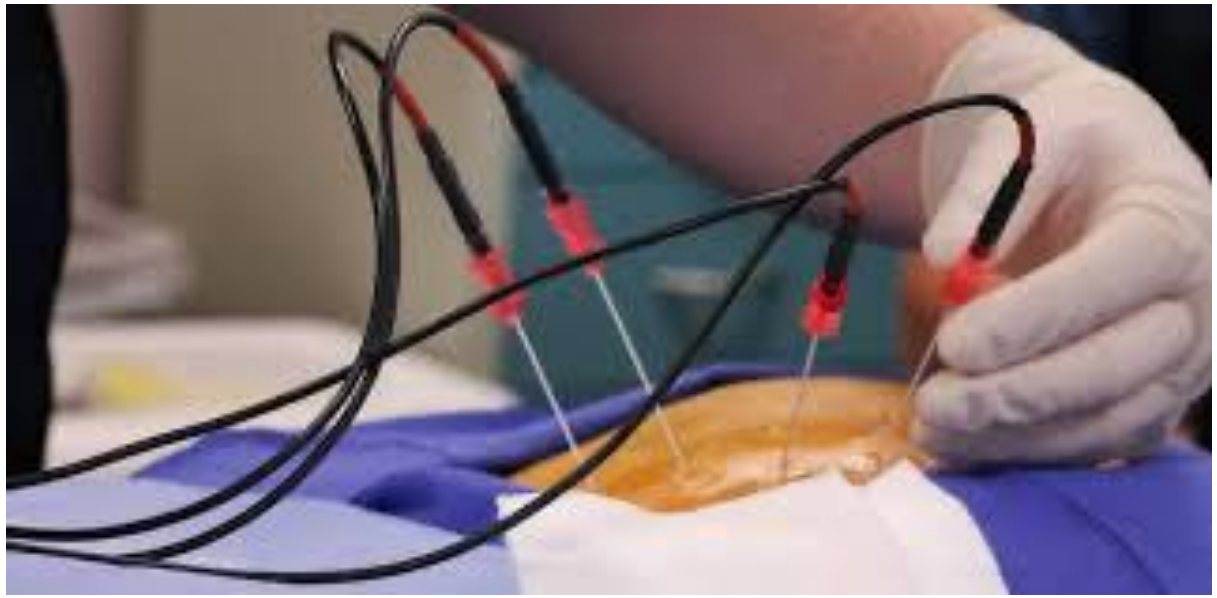


Toxicity:

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

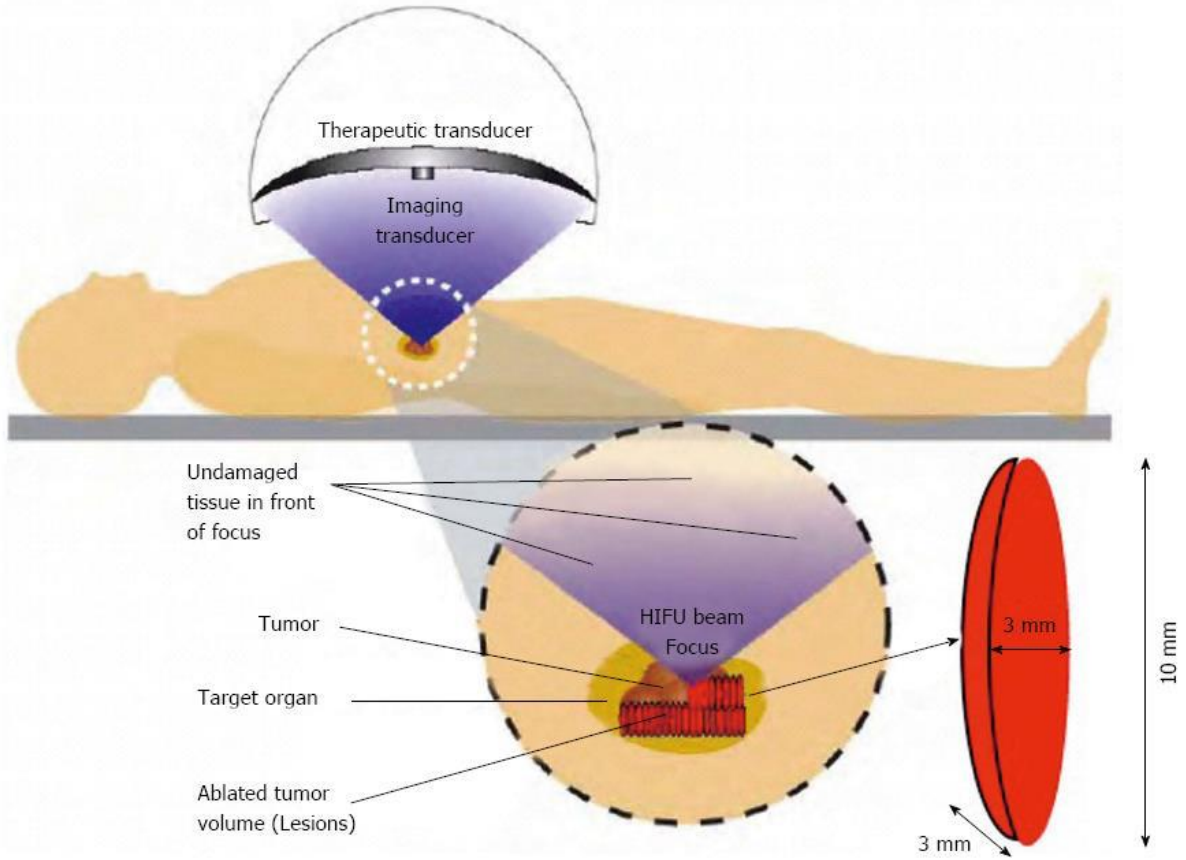
Microwave and Radiofrequency Ablation





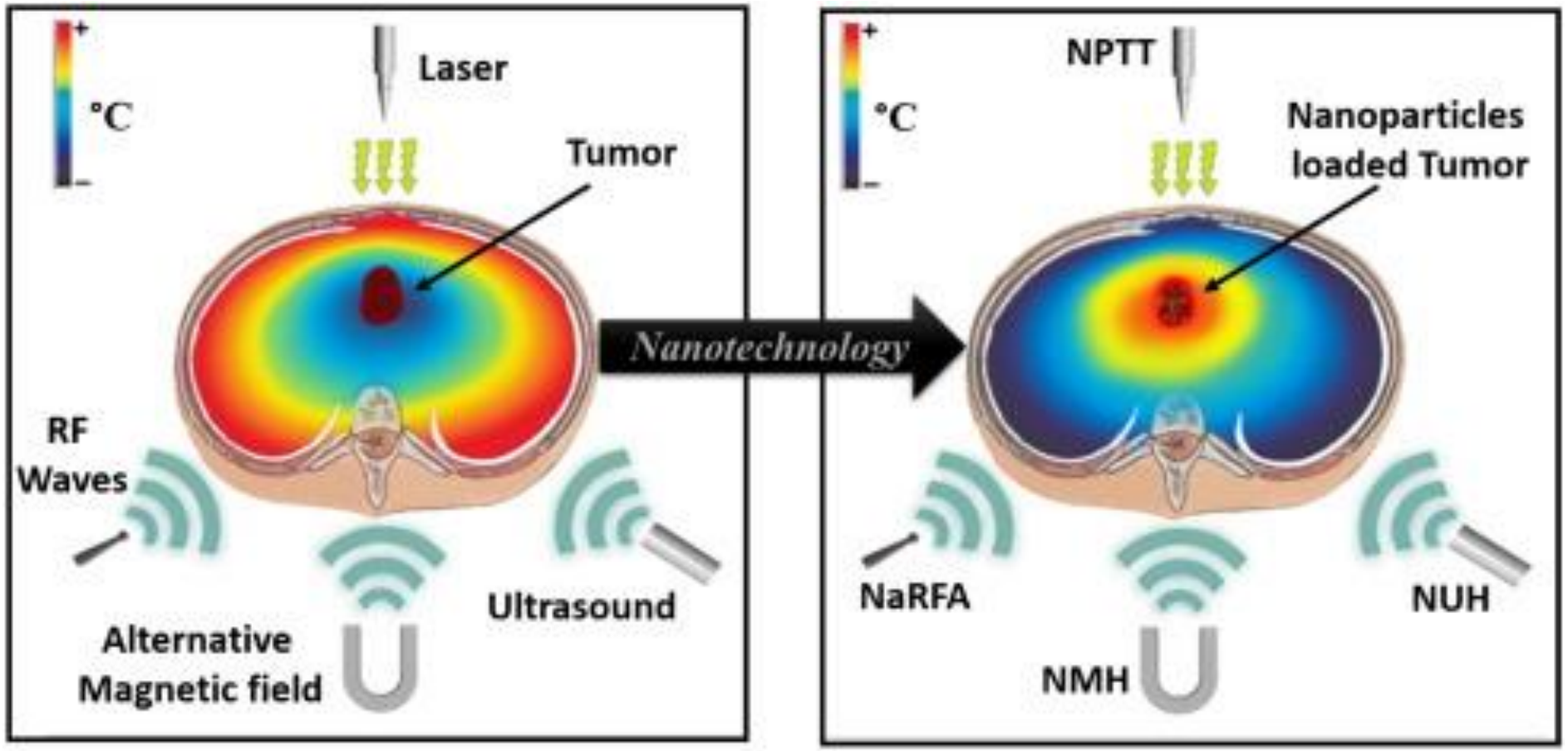
- Tumor entered with thin needle and probe
- Apply electrical current (radiofrequency) or microwave energy
- Tumor necrosis induced
- Residual scar left behind

High-intensity Focused Ultrasound



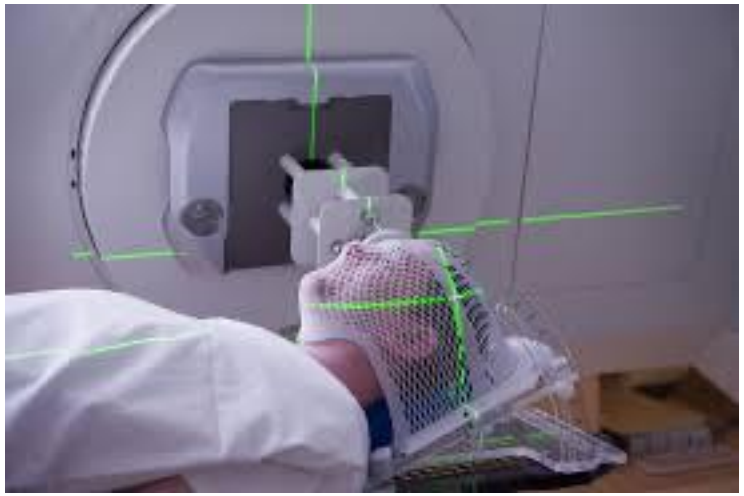
- Non-invasive therapeutic technique
- Uses lower frequency and continuous waves
- Induces thermal damage in tissue (65-85 °C)
- Pulsed waves induce mechanical damage
- Can use with ultrasound or MRI imaging
- HIFU approved in U.S. for prostate cancer treatment in 2015
- Many other tumors under study

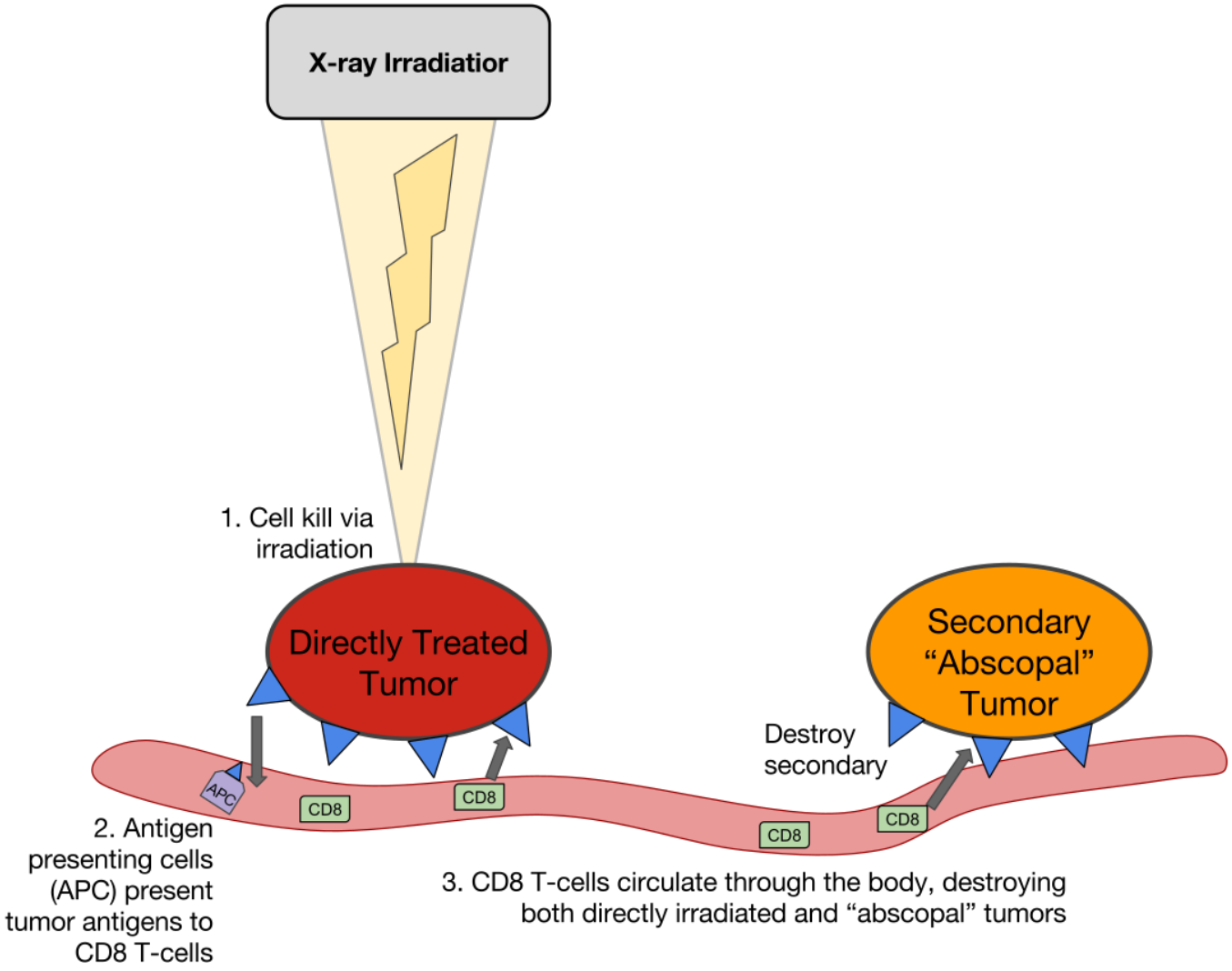
Hyperthermia



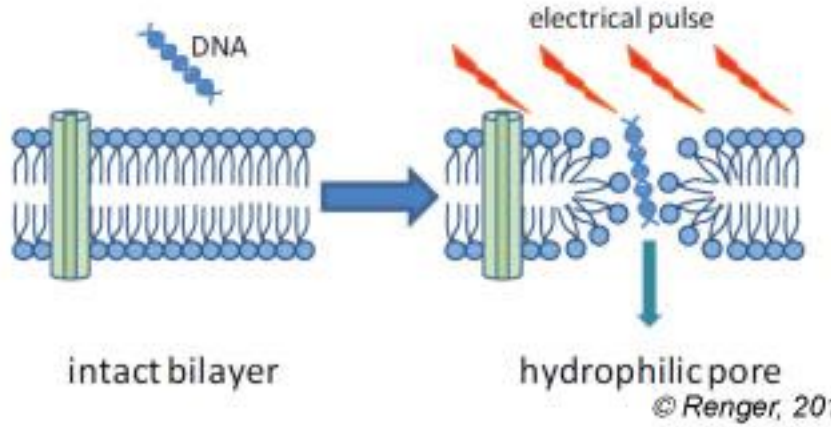
Beik et al. J Controlled Rel 2016

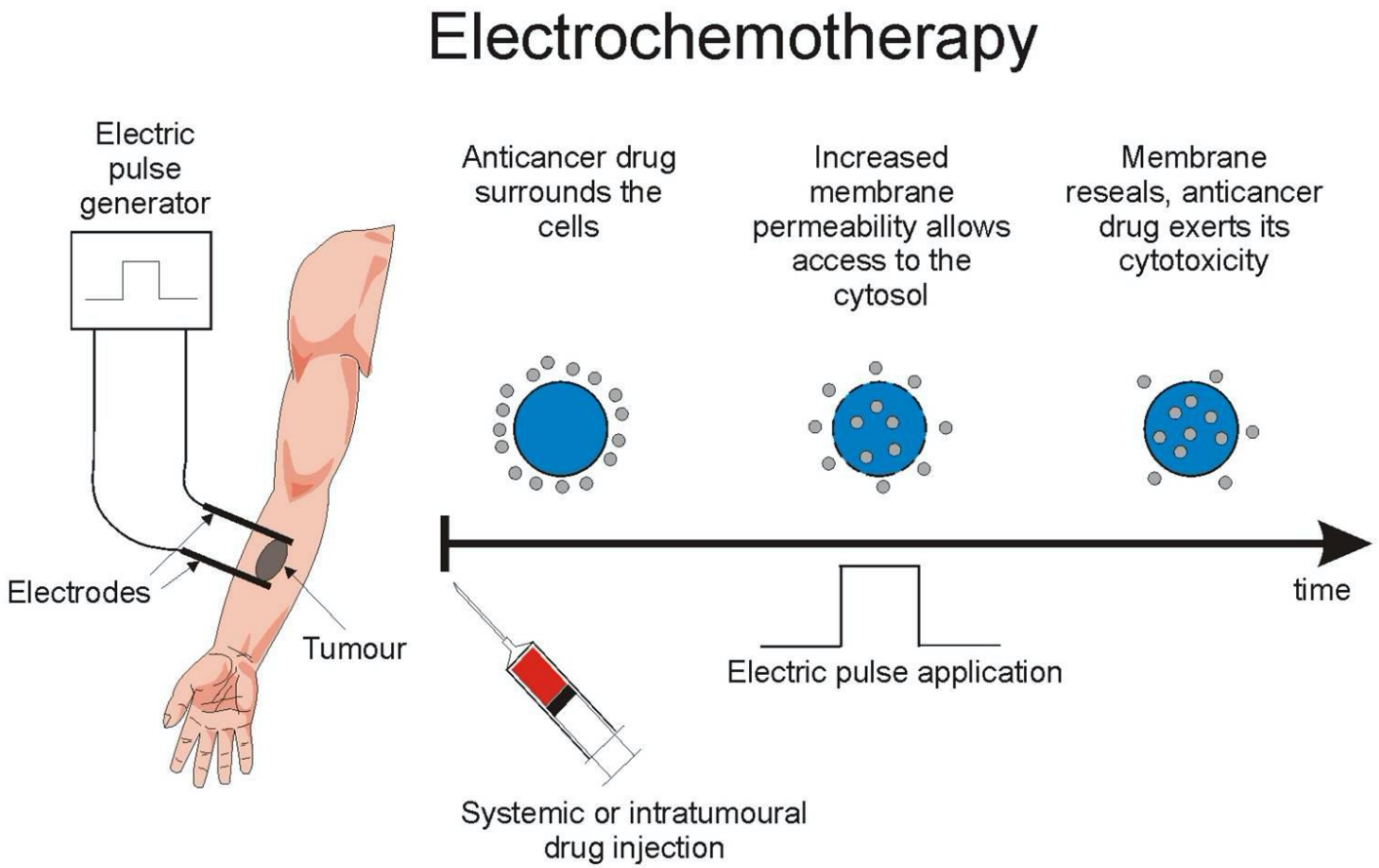
Radiation Therapy

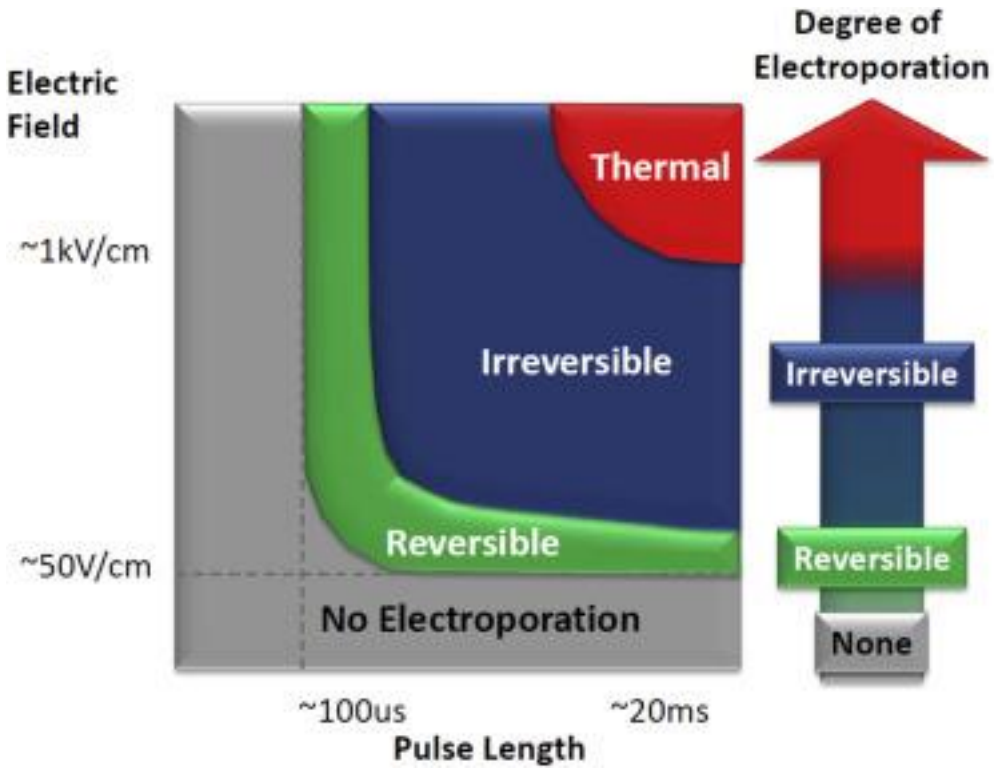




Electroporation

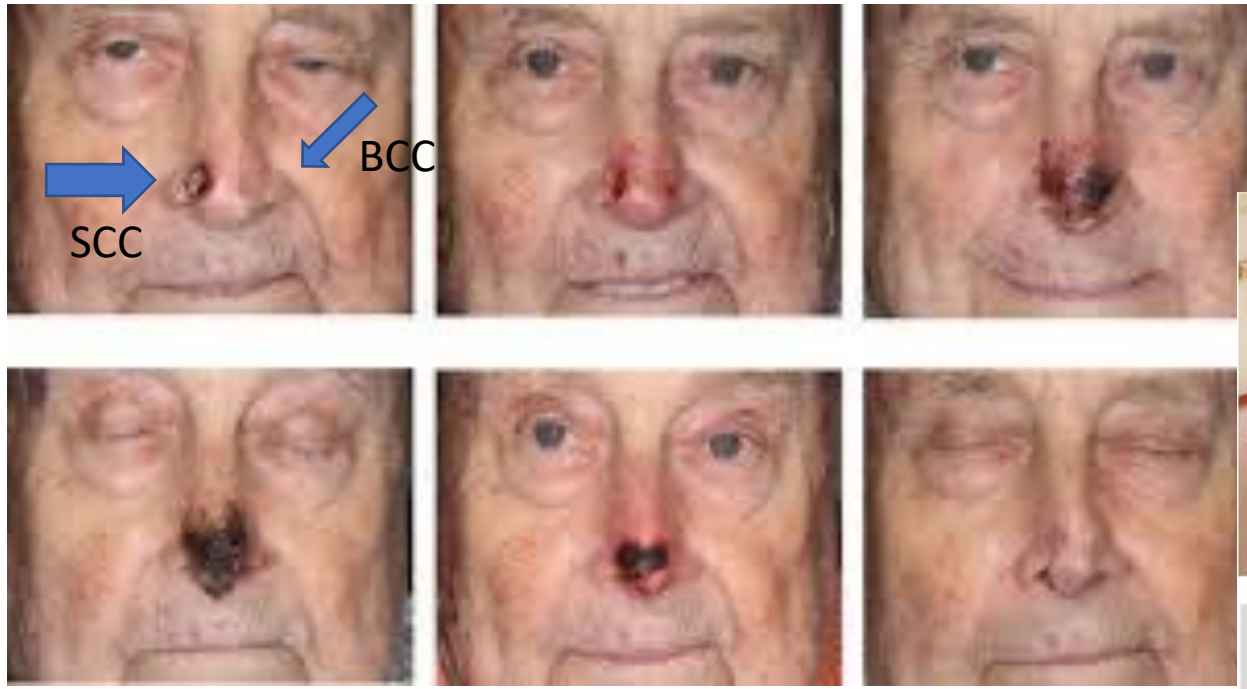




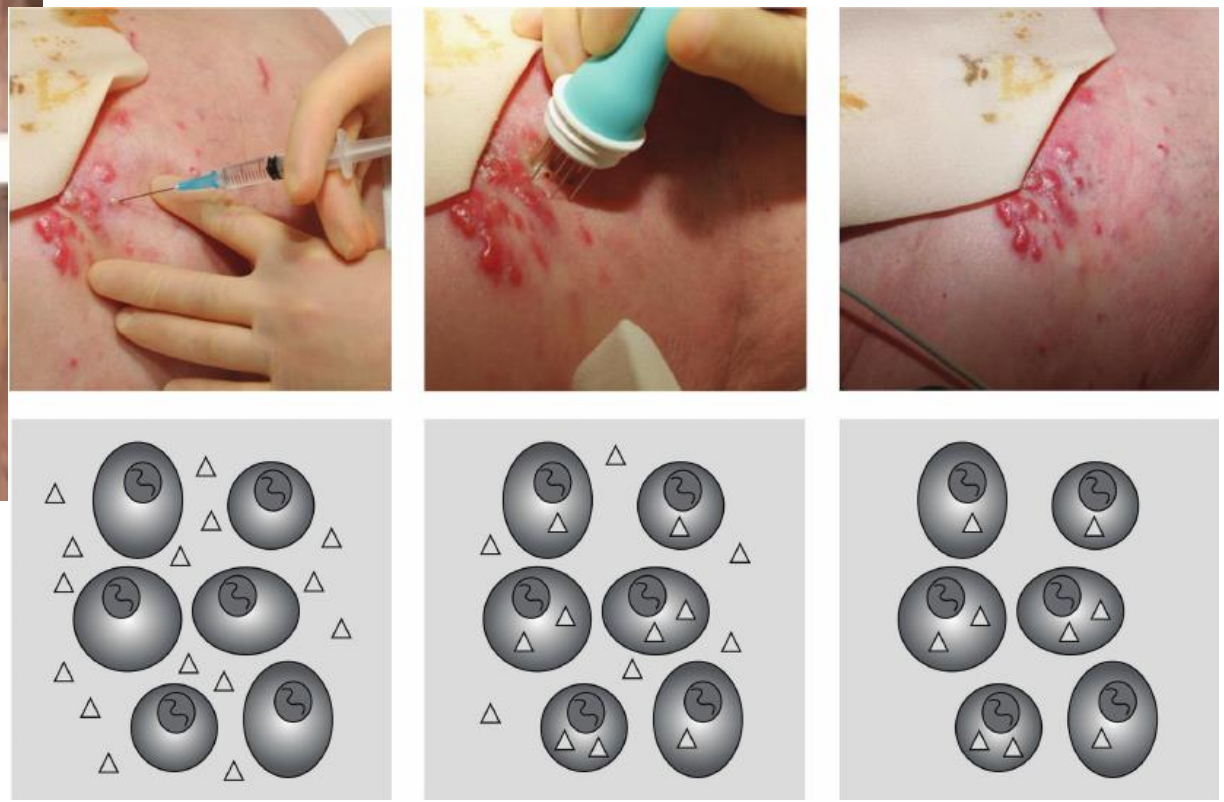


Drug-related Intratumoral Therapy

Intratumoral chemotherapy and electrochemotherapy



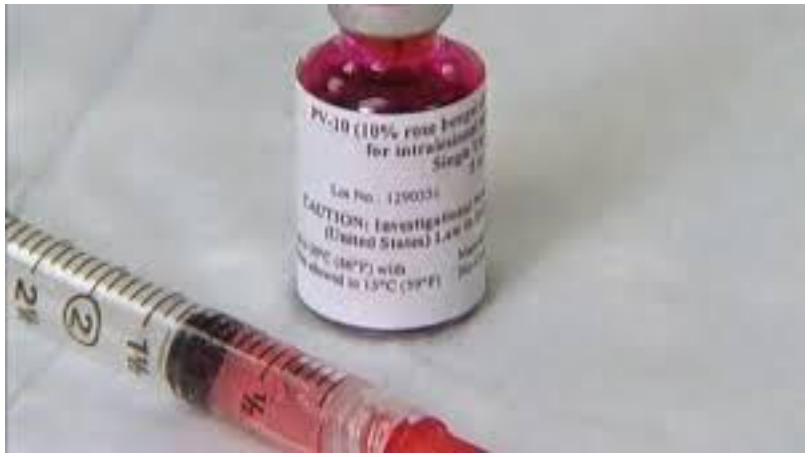
Treated with six weekly intra-lesional injections of 5-FU



Electrochemotherapy with bleomycin

Courtesy Julie Gehl

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

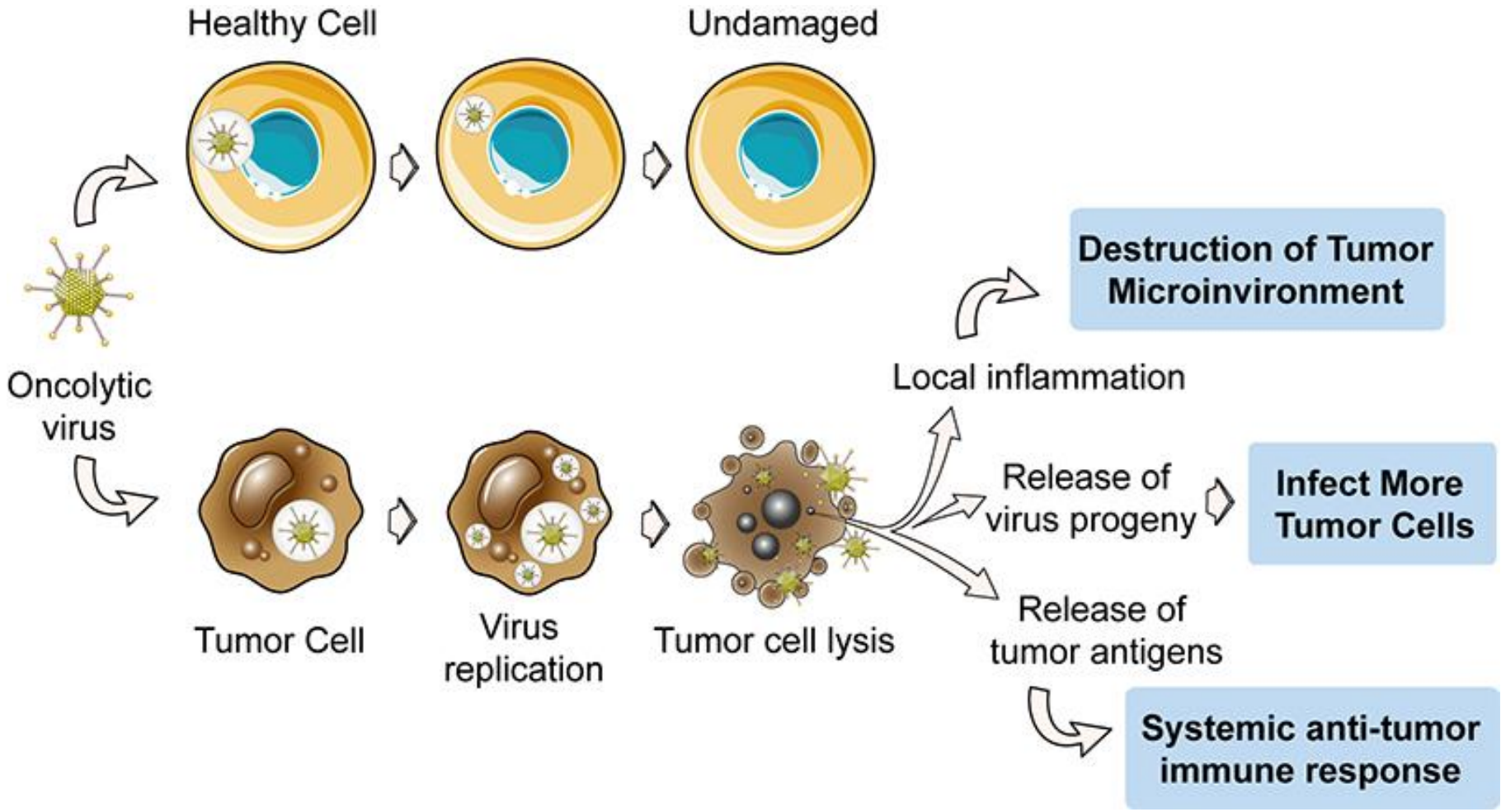
In-transit mets
45 patients

- 87% ORR
- 42% CR

Read et al. J Surg Oncol 2018

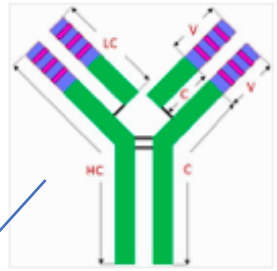
Oncolytic Viruses

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

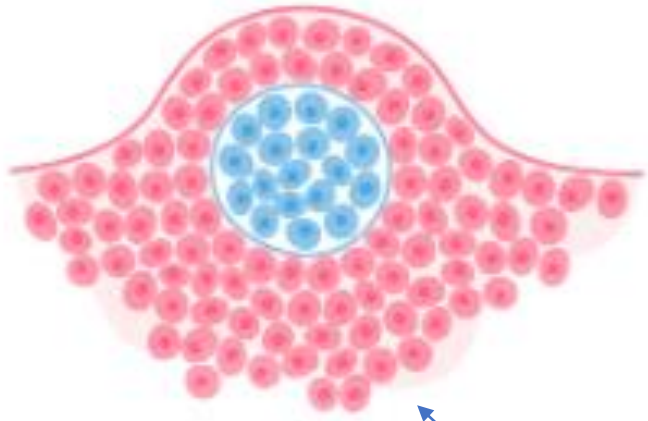


Direct injection of IO Agents into the TME

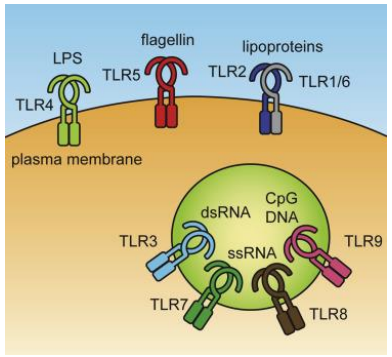
Cytokines



Immune checkpoint inhibitors

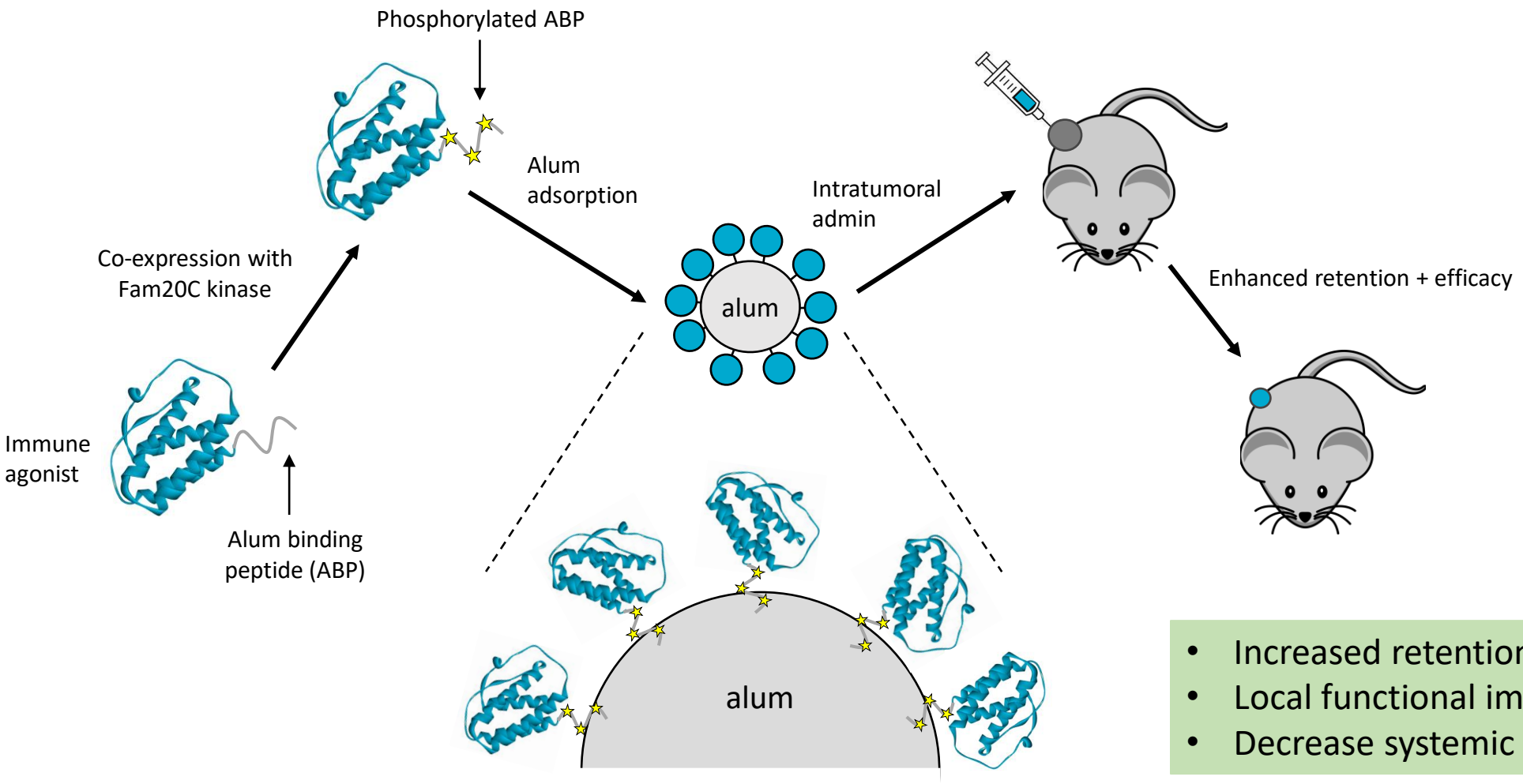


Cell therapy



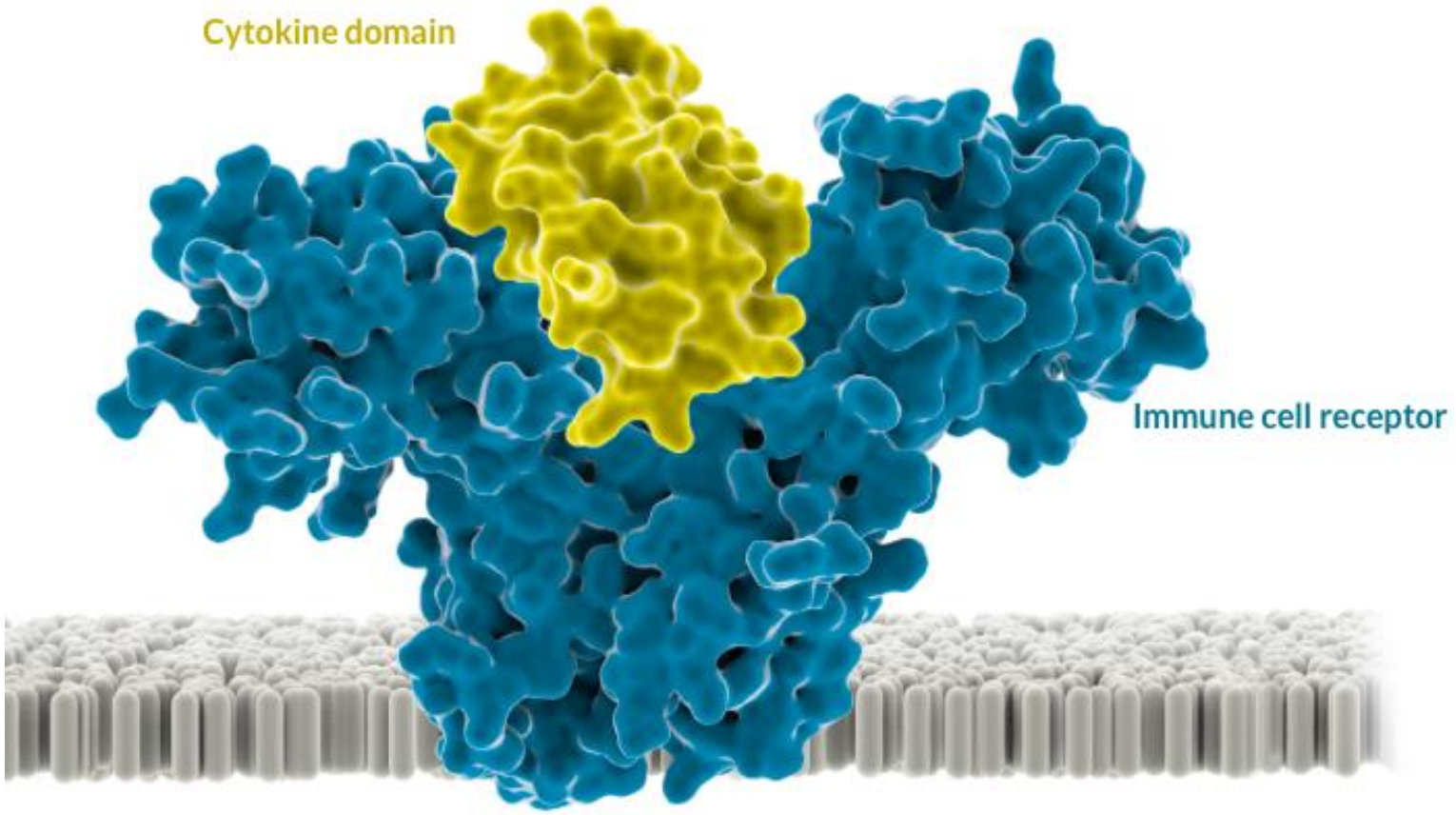
STING and TLR agonists

Delivering IO through scaffolding platforms



- Increased retention at tumor site
- Local functional immune activity
- Decrease systemic toxicity

Masked IO Delivery



Intratumoral Immunotherapy

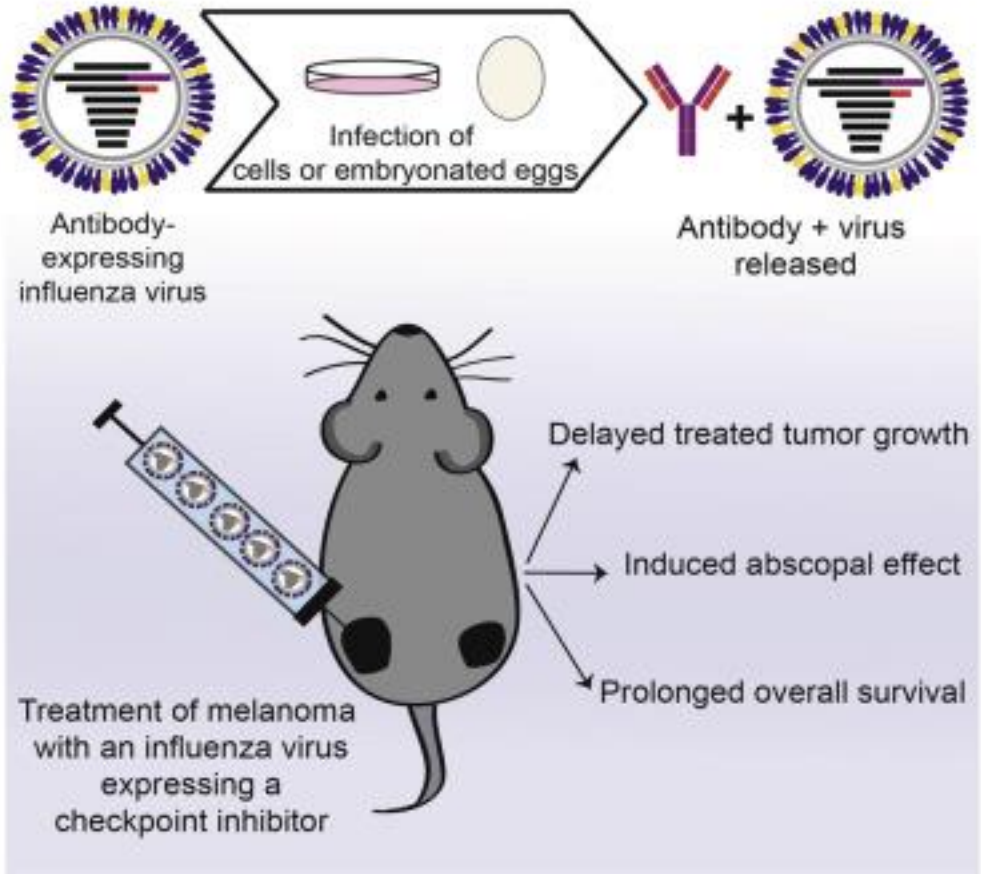
Special Considerations

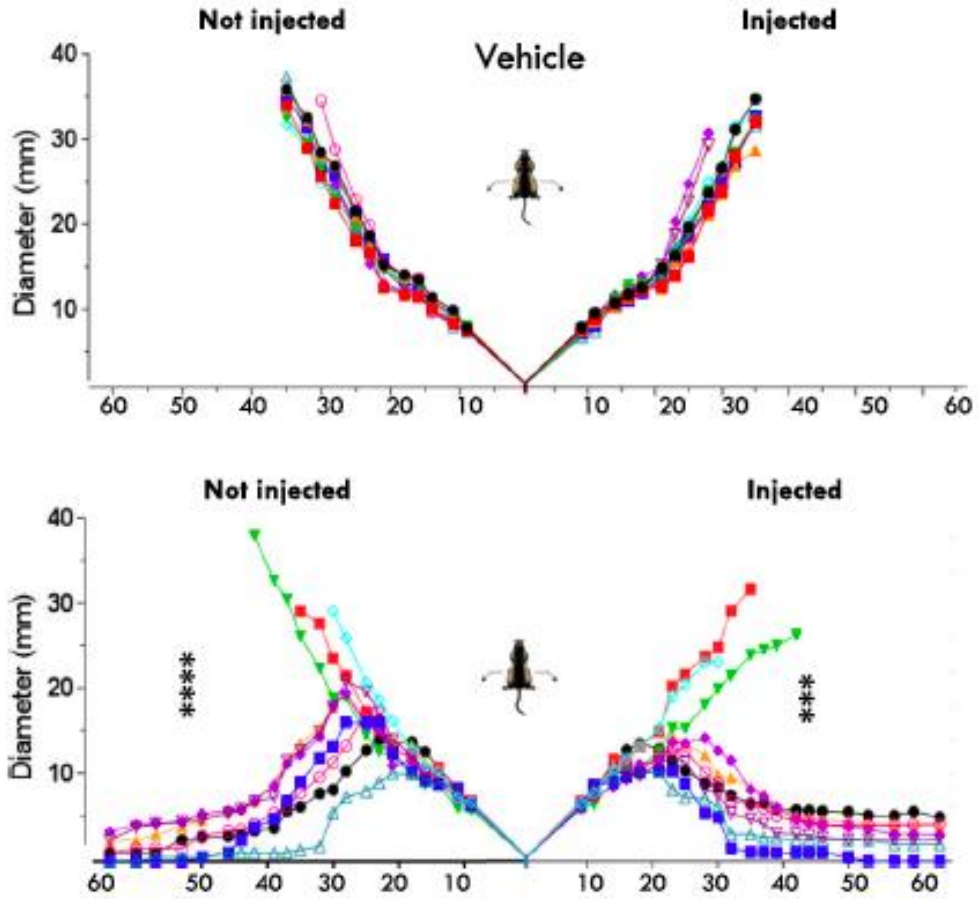
- Pre-clinical Issues
- Clinical Issues
- Logistical Issues

Pre-clinical Issues with Intratumoral Therapy

- 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/anenestic effect?)
- Dose-response relationships should be defined
  - Anti-tumor vs. anti-viral immunity
- Dosing schedule and routes are important to validate

Intratumoral therapy should report injected and un-injected tumor responses





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

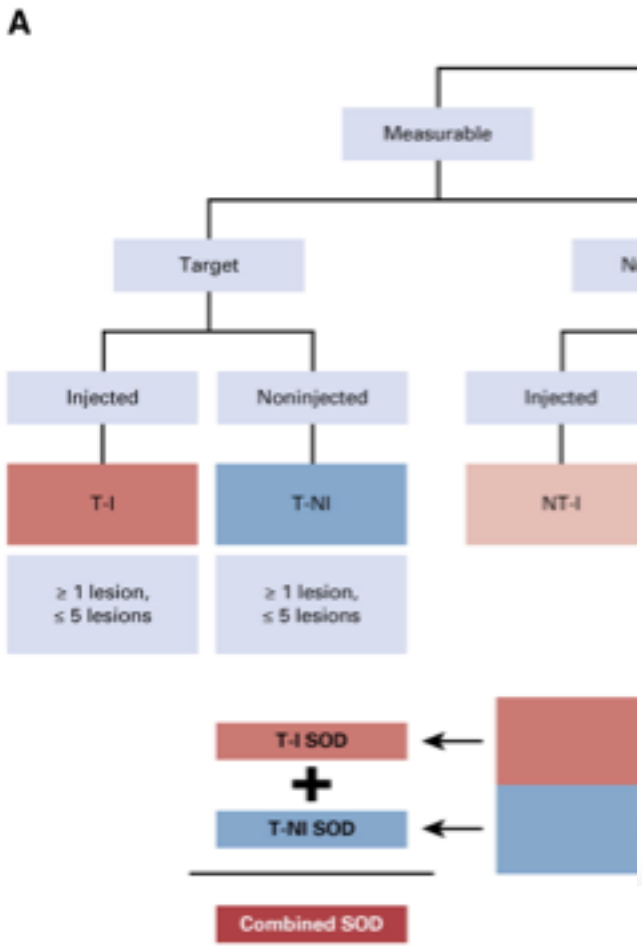
Clinical Issues associated with intra-tumoral immunotherapy

- 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)

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

Alternative Endpoint Assessments: Intratumoral RECIST (itRECIST)

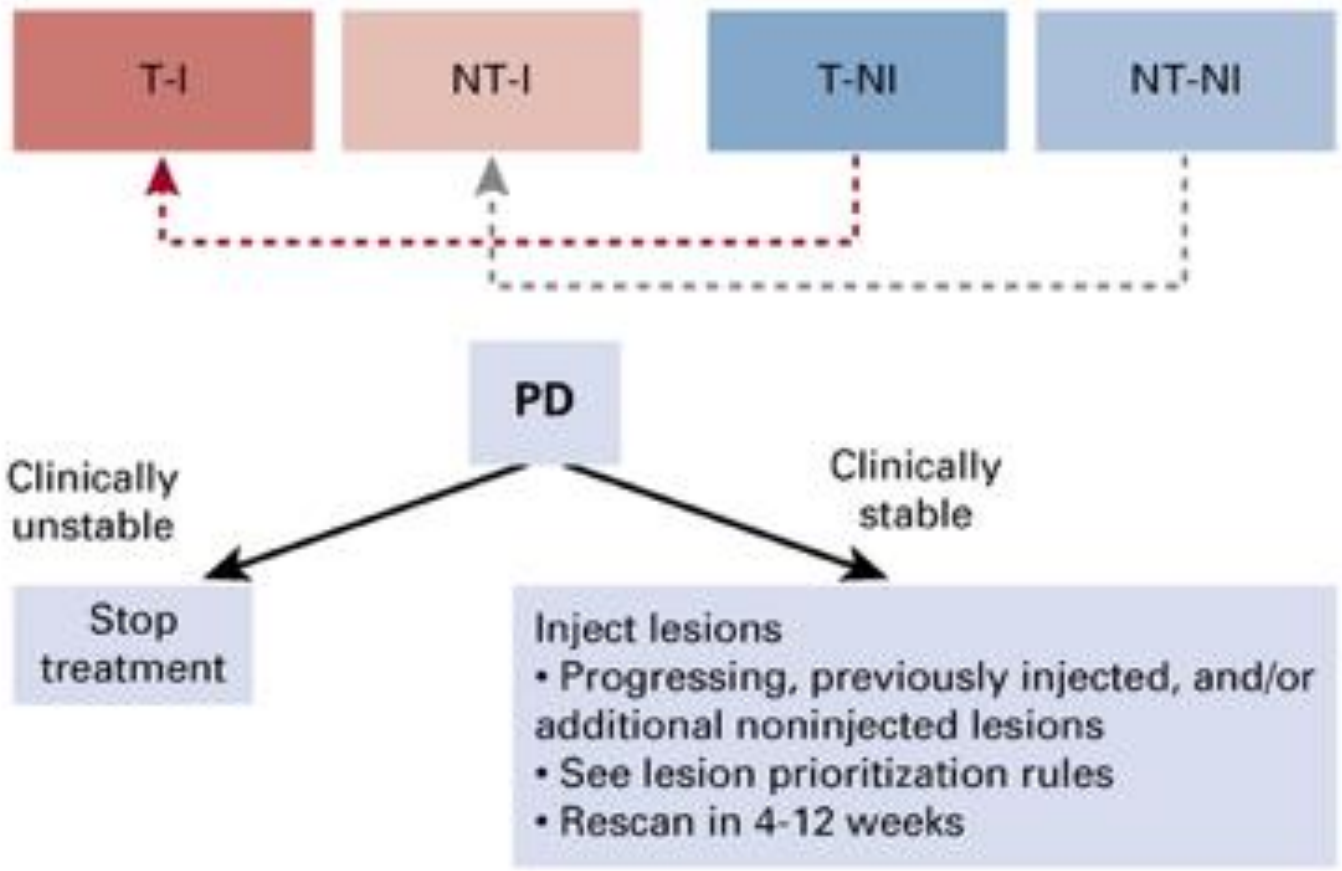


Response	Definition
T-I lesions	
CR	All nonnodal lesions gone, nodal lesions < 10 mm
PR	≥ 30% decrease in SOD from last imaging assessment
PD	≥ 20% increase in SOD from last imaging assessment (≥ 5 mm absolute)
SD	Not enough growth for PD
	Not enough shrinkage for PR
NE	≥ 1 lesion cannot be measured
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. J Clin Oncol. 2020

Treatment beyond progression



Goldmacher et al. J Clin Oncol. 2020

Intravenous delivery of IT agents

Table 1 Selected studies of intravenous oncolytic virus delivery

Study	OV species	Tumors targeted	Sample size	Dose range	Treatment schedule	Intratumoral OV analysis	Adverse events
Machiels et al. [6]	Adenovirus	Epithelial adeno Carcinomas	61	1×10^{10}–1×10^{13} vp	Days 1, 3 and 5 weekly and every 3+week schedule	One patient with colorectal cancer abdominal wall metastasis sample was + by IHC and qPCR 39 days after treatment	Hypoxia, lymphopenia, neutropenia
Nemunaitis et al. [8]	Adenovirus (ONYX-015)	Solid tumors metastatic to lung	10	2×10^{10}–2×10^{13} vp	Weekly in 21 day cycle	qPCR and IHC Virus seen in one tumor biopsy	flu-like symptoms, transient transaminitis
Hamid et al. [9]	Adenovirus	Metastatic colorectal cancer	18	2×10^{12} vp	Every 2 weeks	One autopsy patient with tumor at the root of the mesentery by PCR and IHC	flu-like symptoms, chills, fatigue and lethargy
Rudin et al. [10]	Seneca valley virus	Small cell lung cancer and carcinoid tumors	30	10^{7} – 10^{11} vp	Single dose	qPCR and IHC on autopsy-derived tumor had + IHC for virus	flu-like symptoms
Park et al. [11]	Vaccinia virus-GM-CSF	Treatment-refractory colorectal cancer	15	1×10^{6} – 3×10^{9} pfu	Every 14 days	Plaque assay on plasma and throat swabs	flu-like symptoms
Downs-Canner et al. [12]	Vaccinia virus	Advanced colorectal or other solid cancers	11	3×10^{6}–3×10^{8} pfu	Single dose	qPCR Plaque assay detected 2.5×103 pfu in one patient	fever, chills, abdominal pain, nausea, vomiting, fatigue
Garcia et al. [13]	Adenovirus type 5	Metastatic melanoma	12	1×10^{13} vp	Single infusion	qPCR Viral DNA was only detected in patients treated with doses $>3.3\times10^{11}$	flu-like syndrome fever, chills, neutropenia
Garde-Carbonero, et al. [7]	Adenovirus	Solid adenocarcinomas	17 12 by IV 5 by IT inj.	1×10^{12} vp	Days 1, 3 and 5 followed by tumor resection	Virus hexon protein by IHC found in 10 patients > 80% nuclear staining seen in 21.1% of IT-inj. and 9.4% for IV-inj. Tumor specimens	None
Mell et al. [14]	Vaccinia virus	Head and neck cancer	19	3×10^{6}–3×10^{8} pfu	Day 3 Day 3 and 8 Days 3, 8, 15 and 22 Radiation 33–35 fractions Cisplatin on days 1, 22 and 43	qPCR+ in 5 patients (range 4–409 copies/mg) Virus (2.0×10^{3} pfu) detected in tongue tumor in 1 patient at 7 days	Rigors, fever, fatigue, rash, hypotension, mucositis, nausea, vomiting
Samson et al. [15]	Reovirus	Brain tumors	9	1×10^{10} $TCID_{50}$	Single one-hour infusion	IHC for reovirus σ3 capsid protein was low in 6/9 tumors Ig TEM + in 9/9 ISH+ 8/9 qPCR+ in 4/7	Lymphopenia, flu-like symptoms

Abbreviations: igTEM immunogold transmission electron microscopy, inj injection, IT intratumoral, IV intravenous, pfu plaque-forming units, qPCR quantitative polymerase chain reaction assay, TCID tissue culture infective dose, vp viral particle

- 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

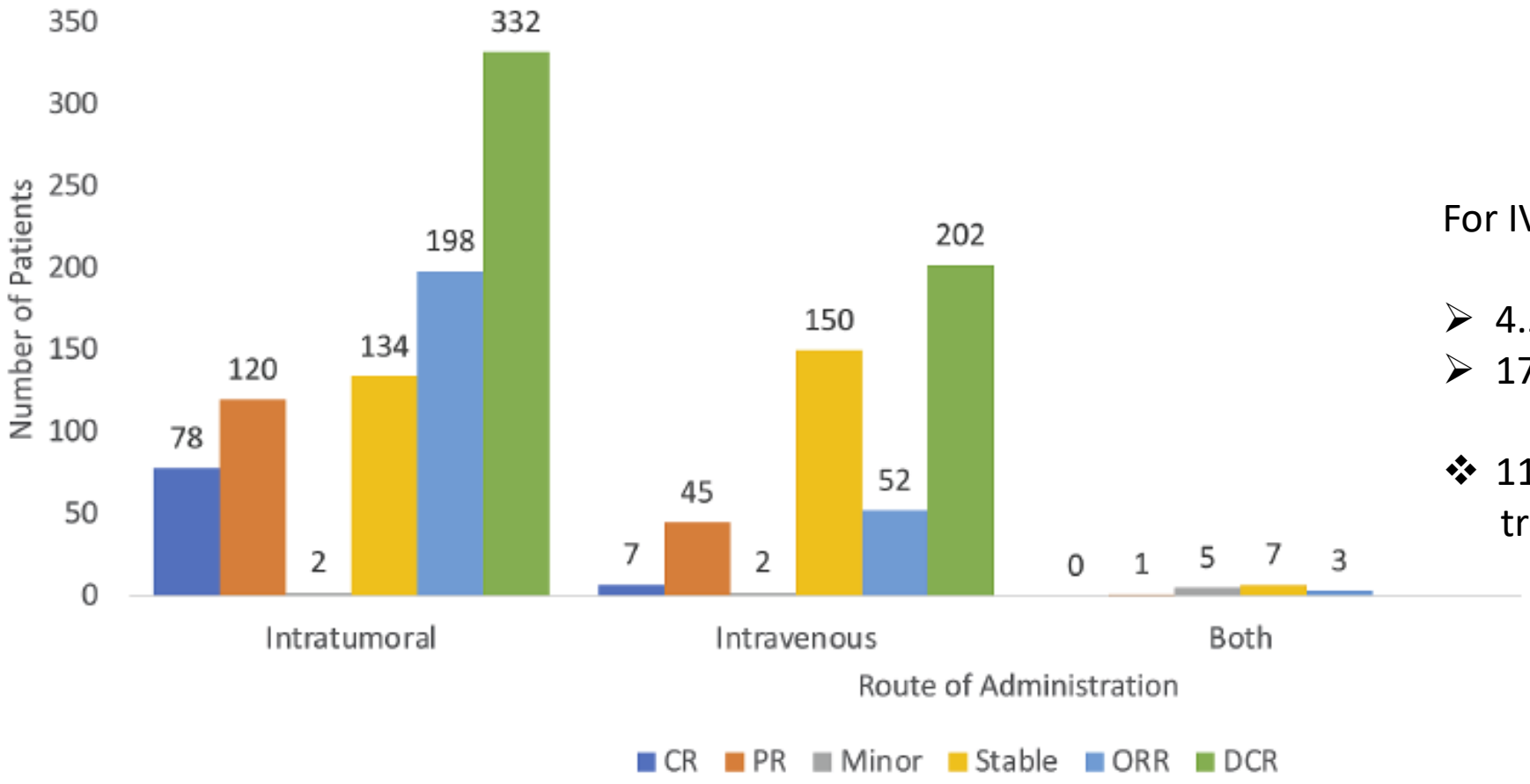
Bommareddy and Kaufman JITC 2019

Objective Clinical Response with OVs by Route of Administration

For IT treatment:

- 13.3% ORR
- 22.4% DCR

- 1482 total IT treated patients



For IV treatment:

- 4.5% ORR
- 17.6% DCR

- 1147 total IV treated patients

Machedo et al. J Immunother Cancer 2021

Conclusions

- Intratumoral immunotherapy is the local delivery of agents that induce anti-tumor immune responses
- There are many types of intratumoral immunotherapy
  - Physical approaches
  - Drug-based approaches
  - IV delivered and locally activated
- There are unique pre-clinical, clinical and logistical considerations associated with intratumoral immunotherapy
- Rational combination approaches in development
  - Neoadjuvant, IO combinations, non-IO combinations