Intratumoral Immunotherapy

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Disclosures

- I am an employee of Immuneering Corporation
- I am a consultant to Replimune, Inc.

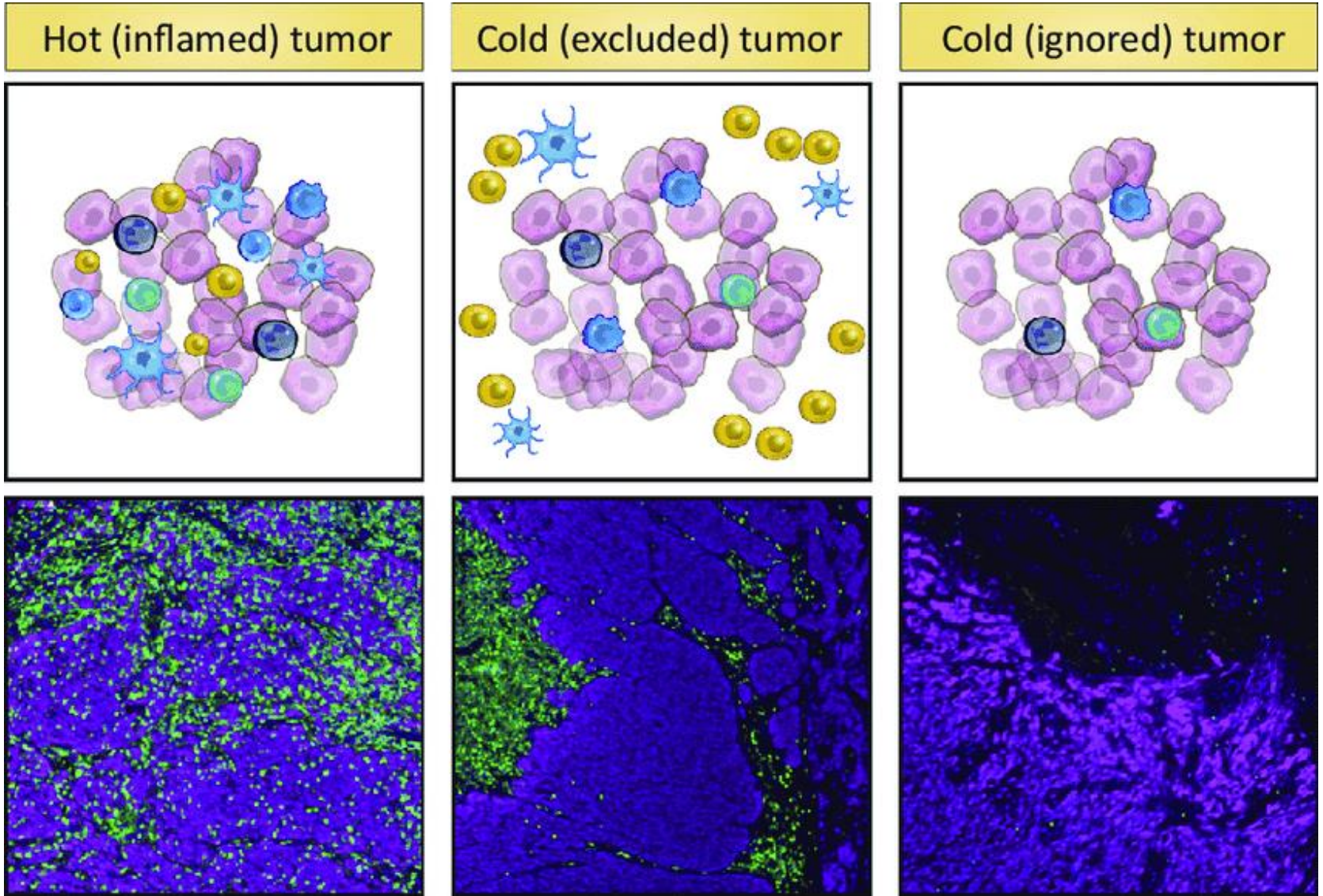
Agenda

- Definition, rationale and history of intratumoral immunotherapy
- Types of intratumoral immunotherapy (ITIT)
  - Physical
  - Drug-related
- Pre-clinical issues
- Clinical and Logistical issues
- Integrating ITIT into combination approaches

Intratumoral Immunotherapy

Definitions and Rationale

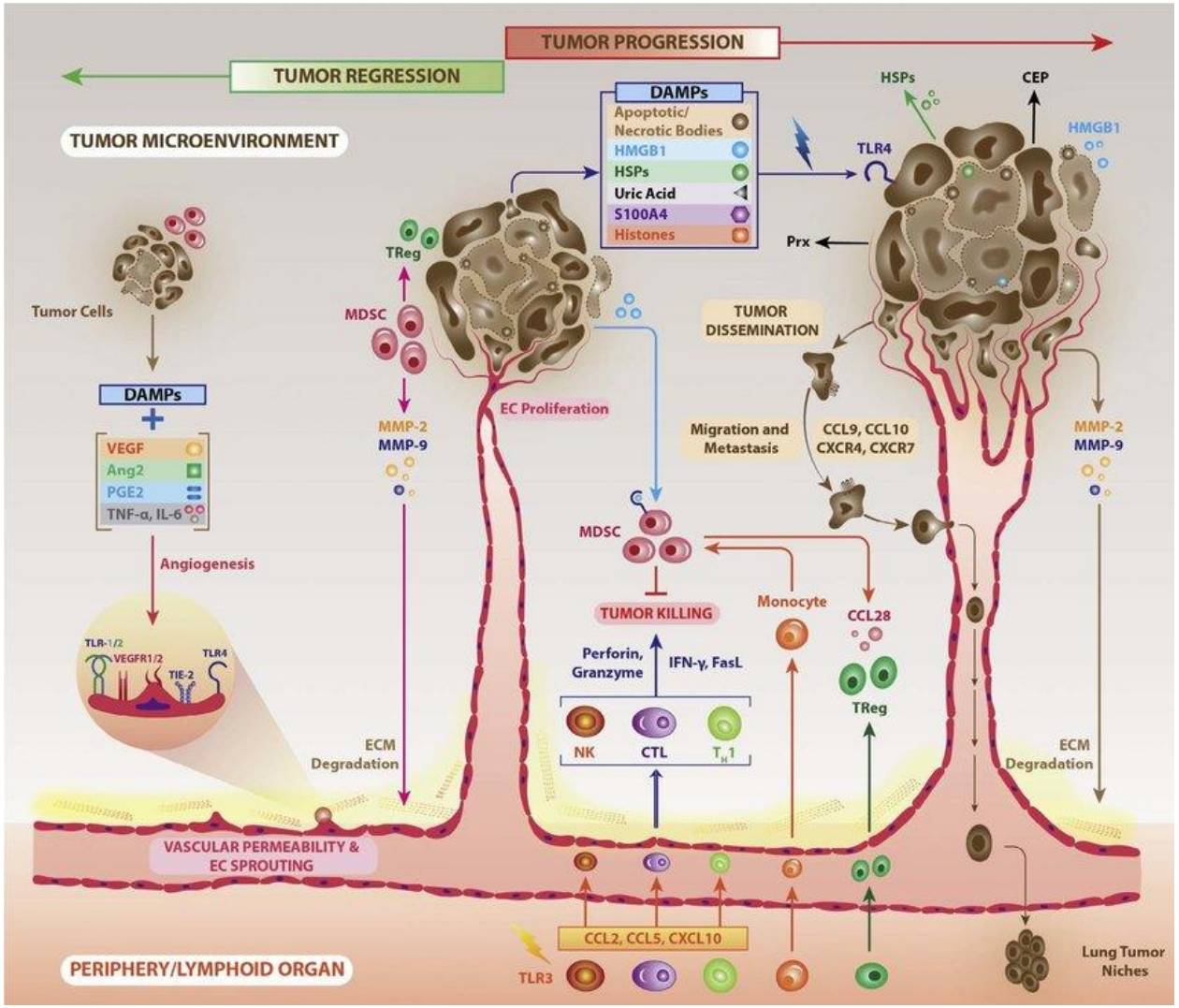
Hot vs. cold tumor microenvironment



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

van der Woude et al. Trends Cancer 2017

Tumors arise in complex – and constantly evolving - microenvironments

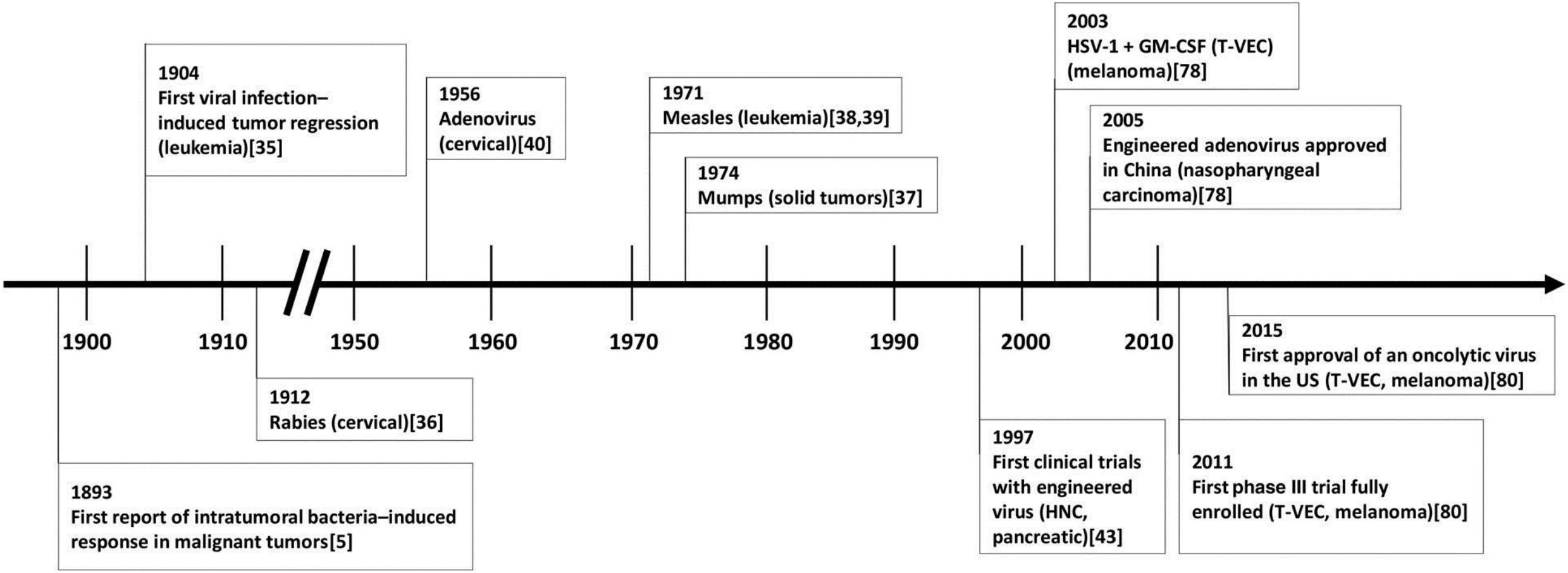


Malinovskaya et al. Front Oncol 2019

What is intra-tumoral Immunotherapy?

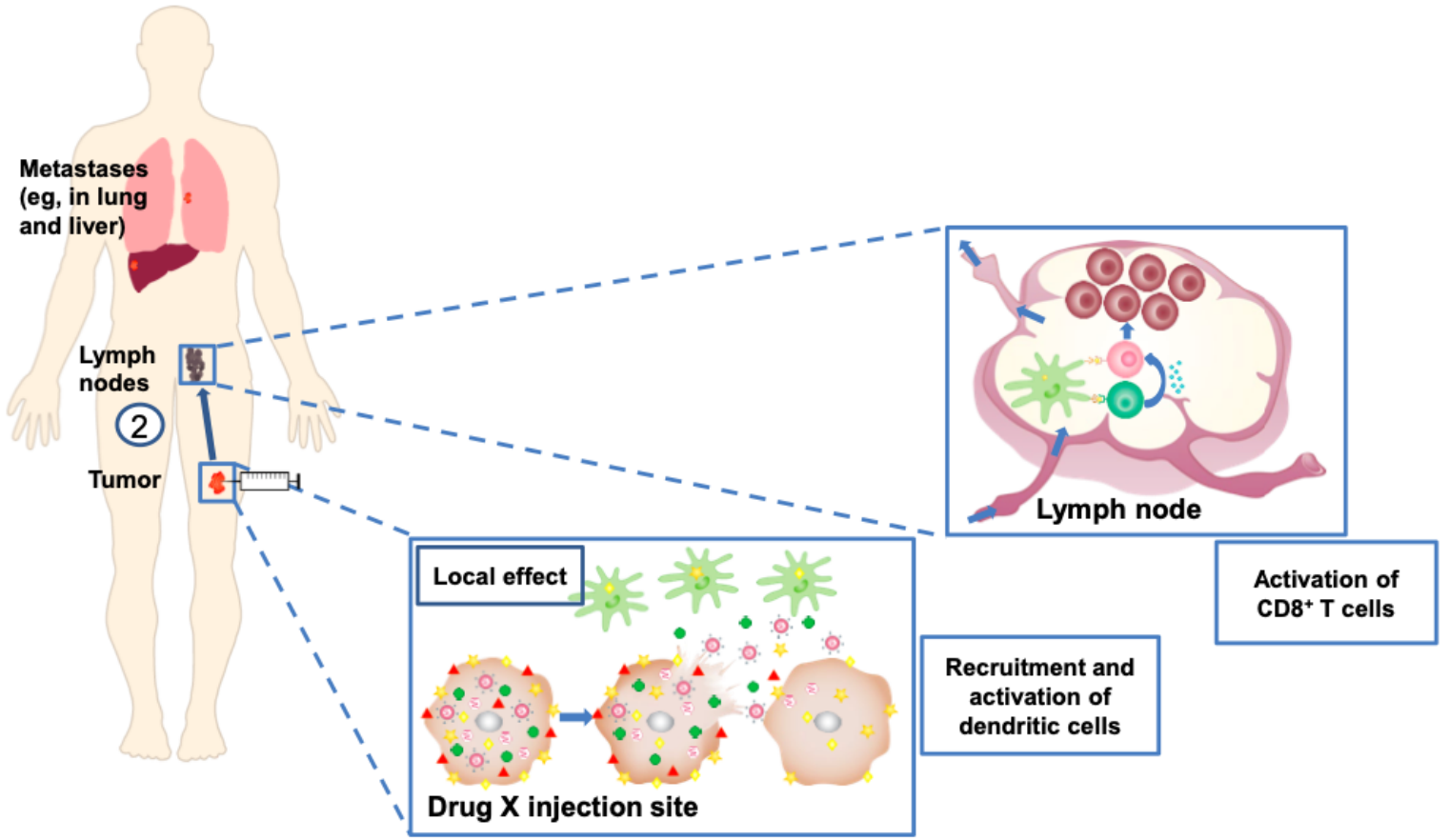
- Therapeutic approach that delivers IO drugs directly into the tumor microenvironment
  - May be physical or chemical
  - Can be given by direct injection; or
  - Regional intra-vascular injection
  - Systemic delivery with local activation in the TME?
- Focuses on generating local immune responses
  - 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



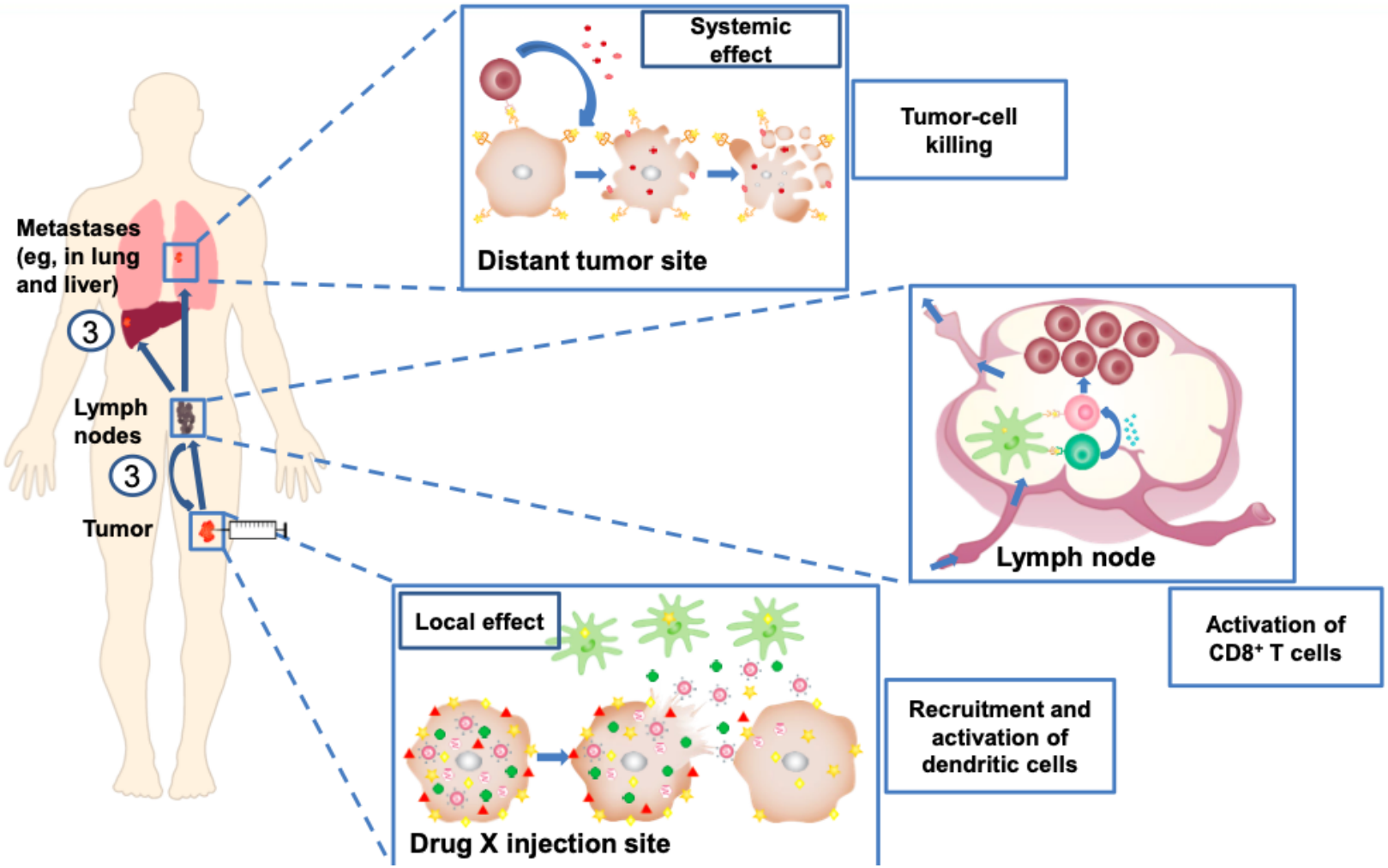
Hamid and Puzanov The Oncologist 2019

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*

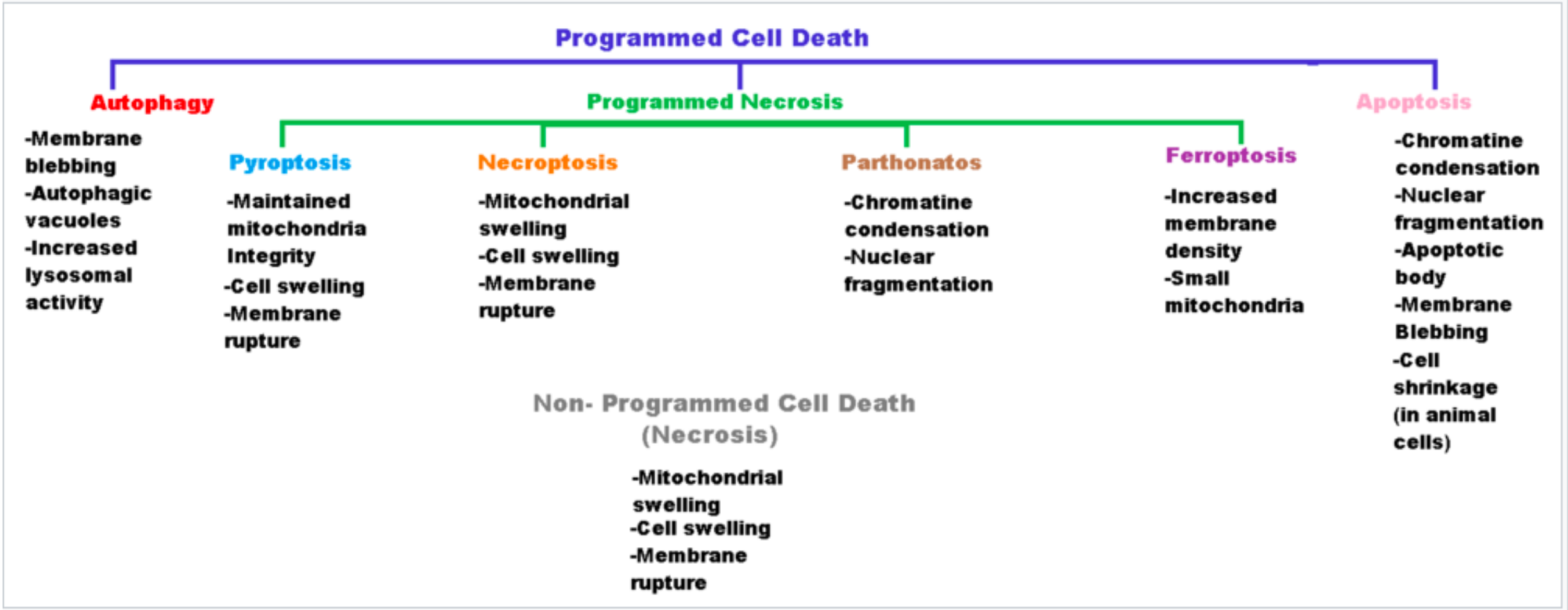
Intratumoral therapy ***may*** induce systemic immunity (i.e. abscopal or anenestic effect)



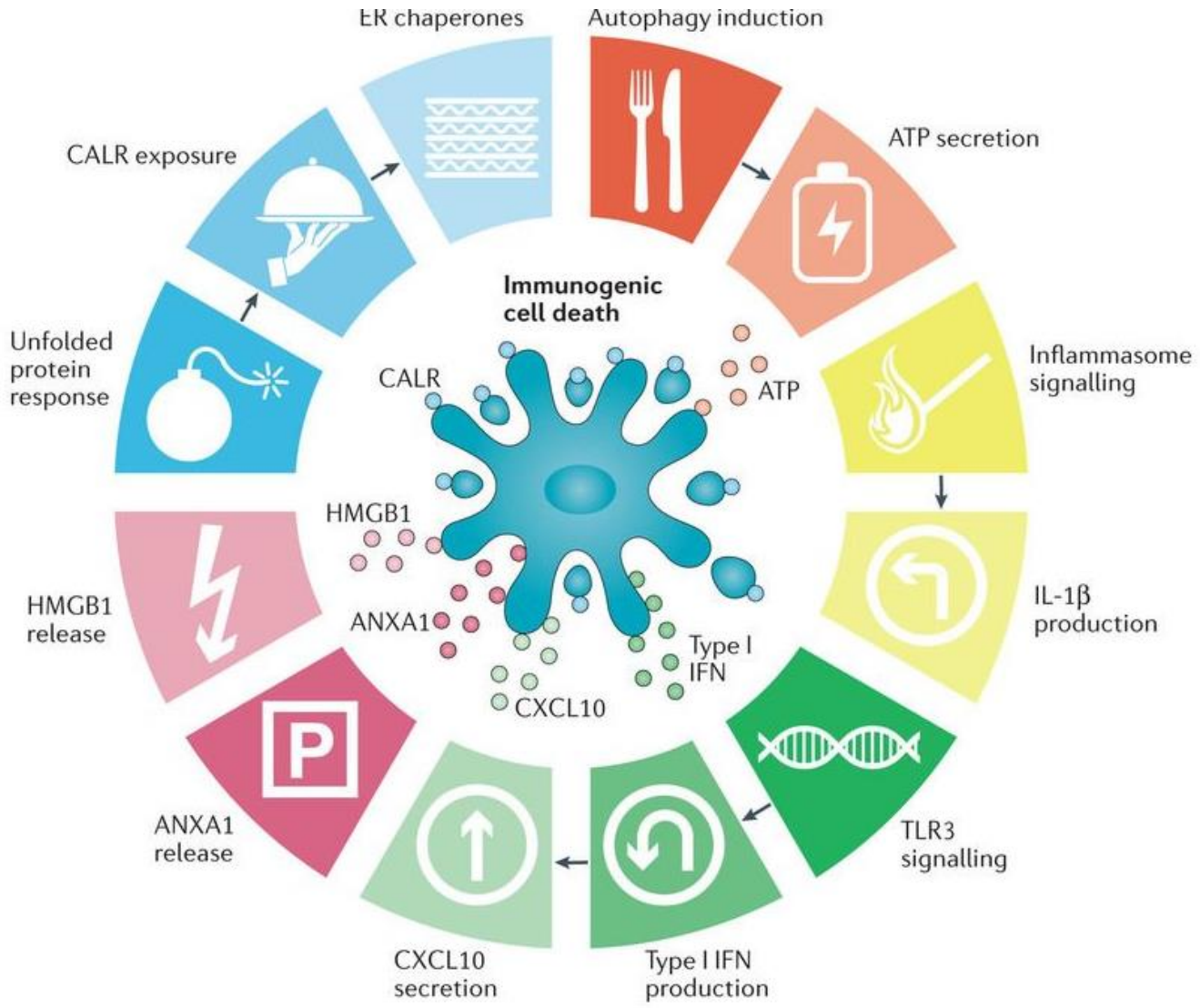
How cells die makes a difference

NECROSIS	APOPTOSIS
Always pathological	May be **physiological** or **pathological**
Affects adjacent group of cells	Affect single cells
Cell size is increased	Cell size is **shrunken**
Passive	**Active**
Causes inflammatory reaction	**No inflammatory** reaction
Plasma membrane is disrupted	Plasma membrane is **intact**

Type of cell death has implications for generating cell-specific immune responses

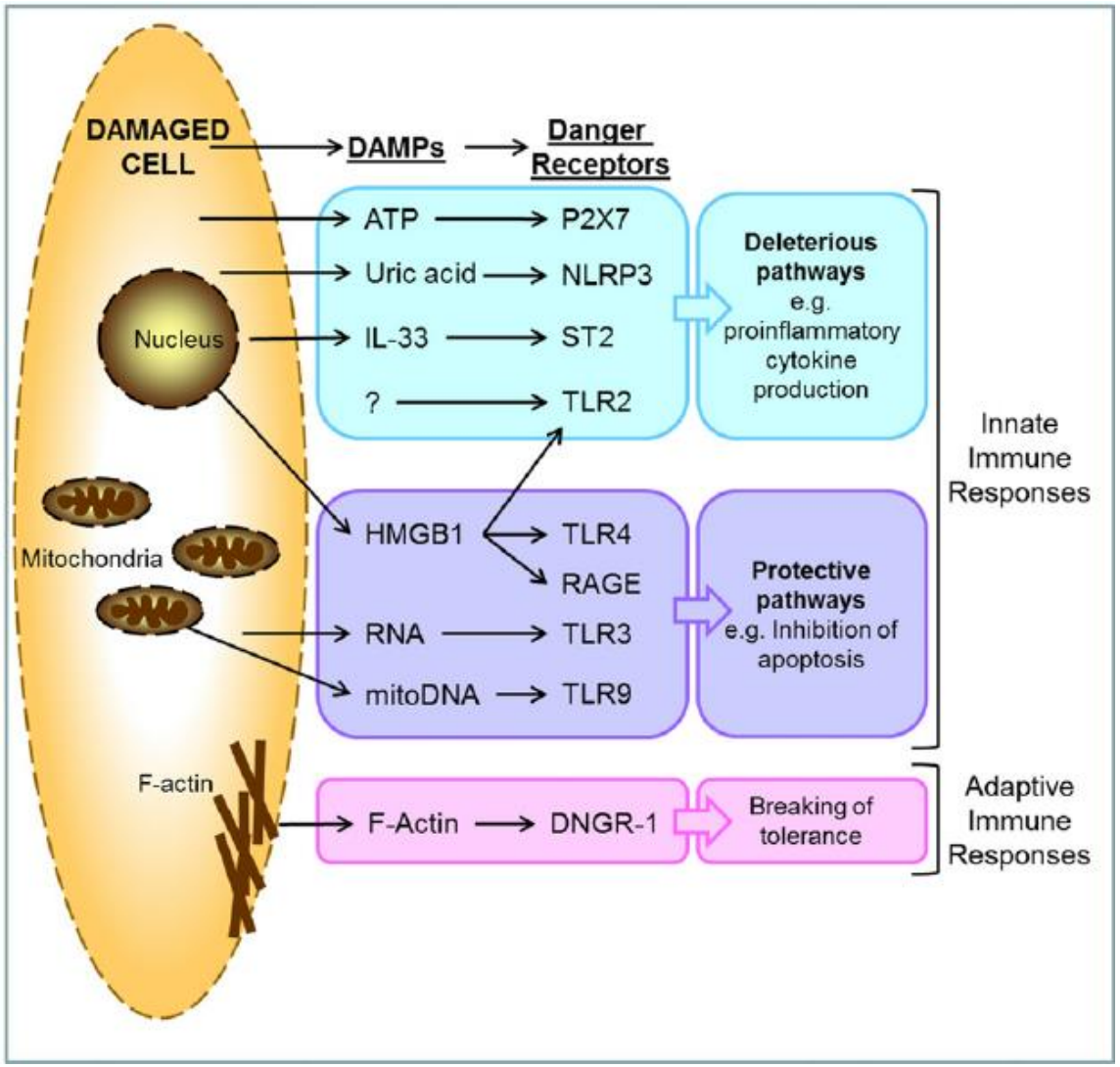


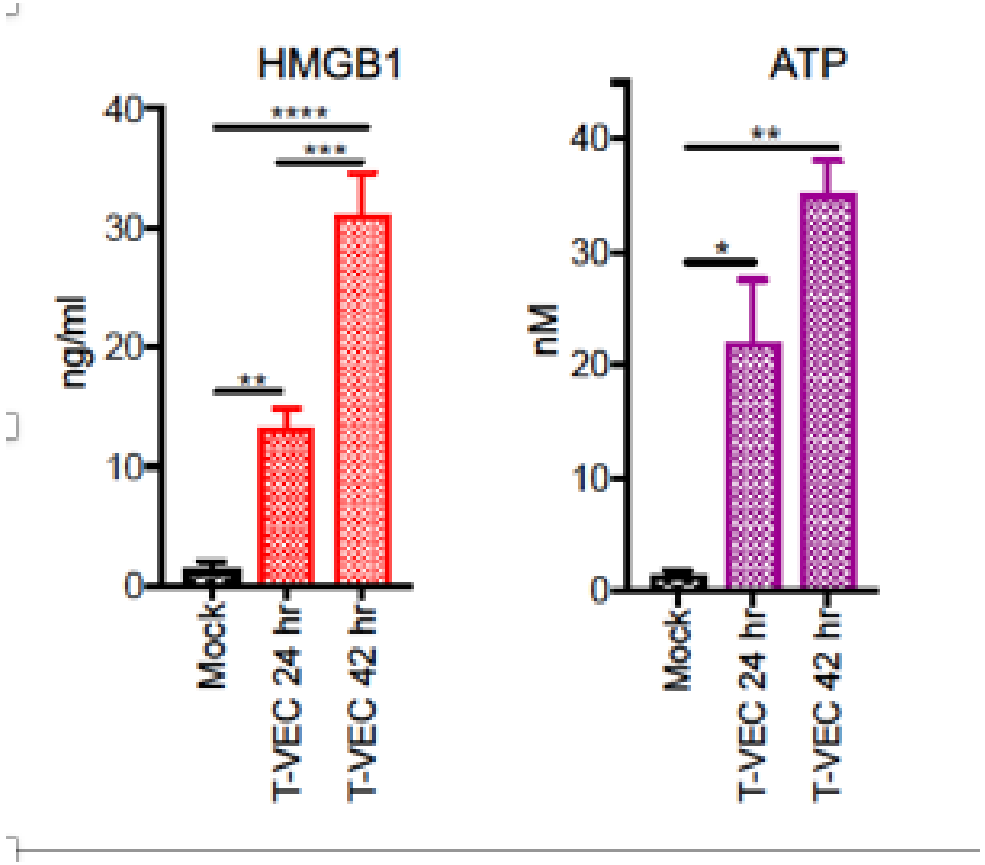
Immunogenic cell death



Galluzzi et al. Nature Immunol. 2017

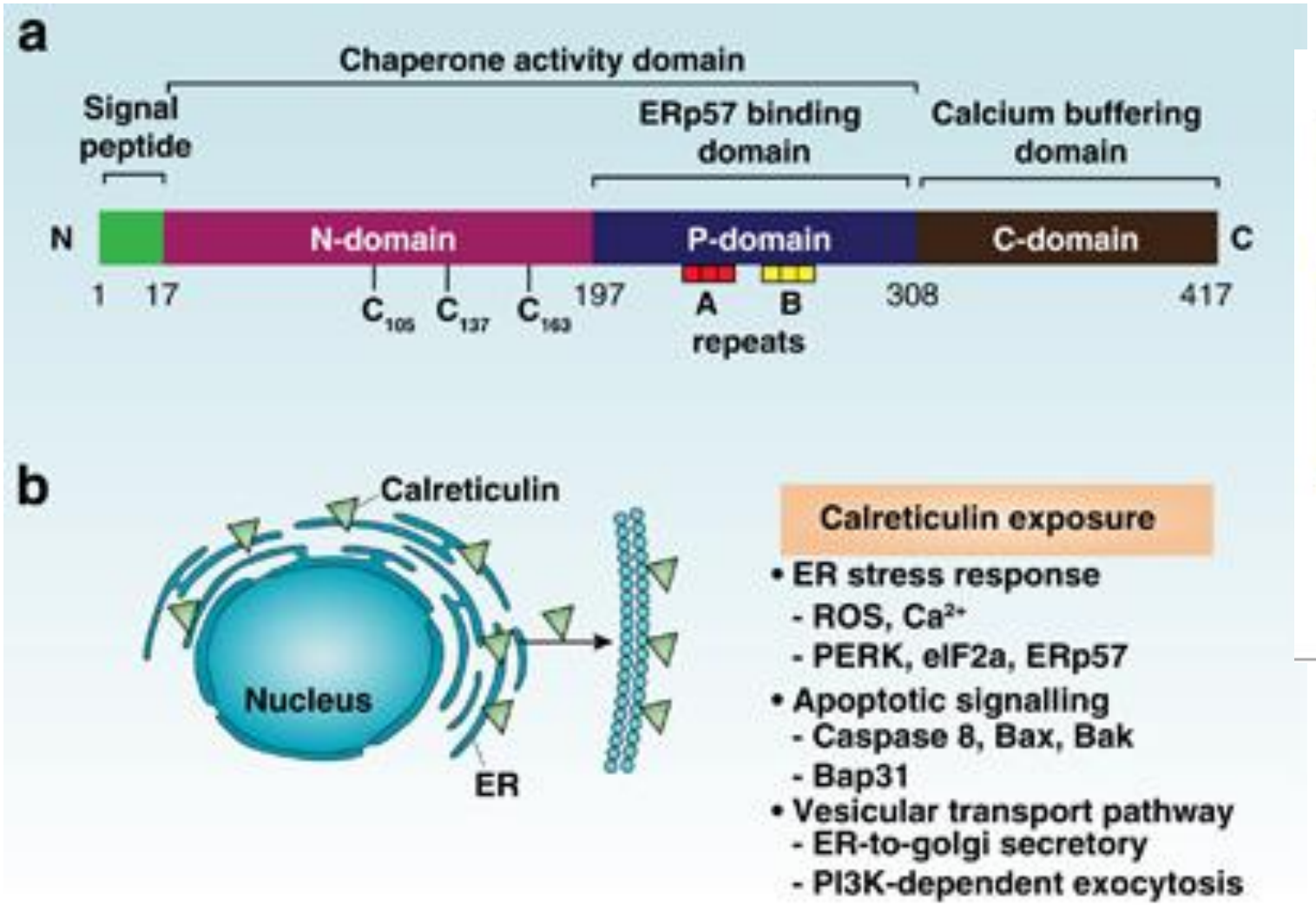
Traditional ICD measured by release of DAMPs

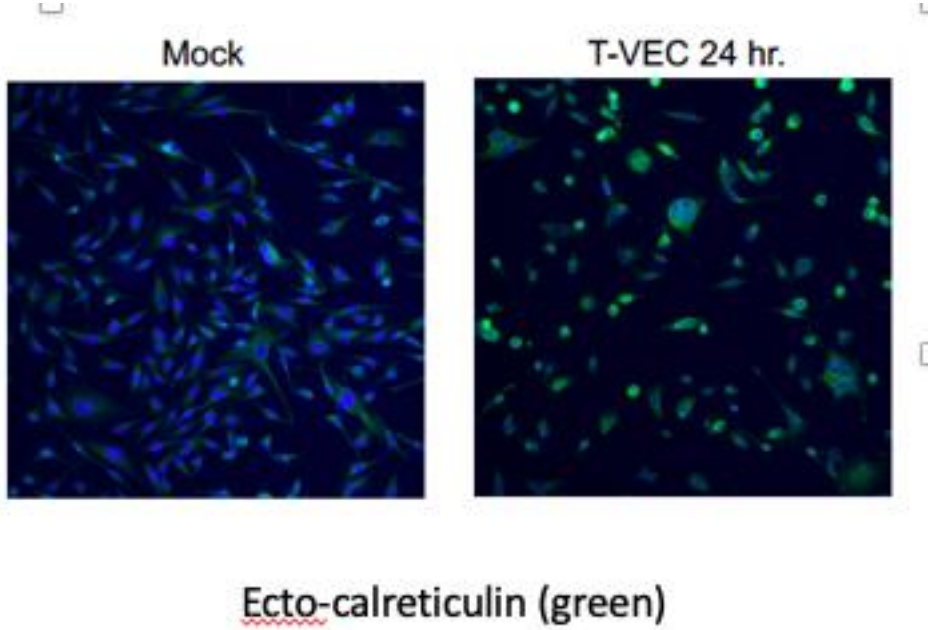




Courtesy Dr. Cory Hogaboam
Bommareddy et al. Oncolimmunol. 2018

Ecto-calreticulin exposure denotes ICD

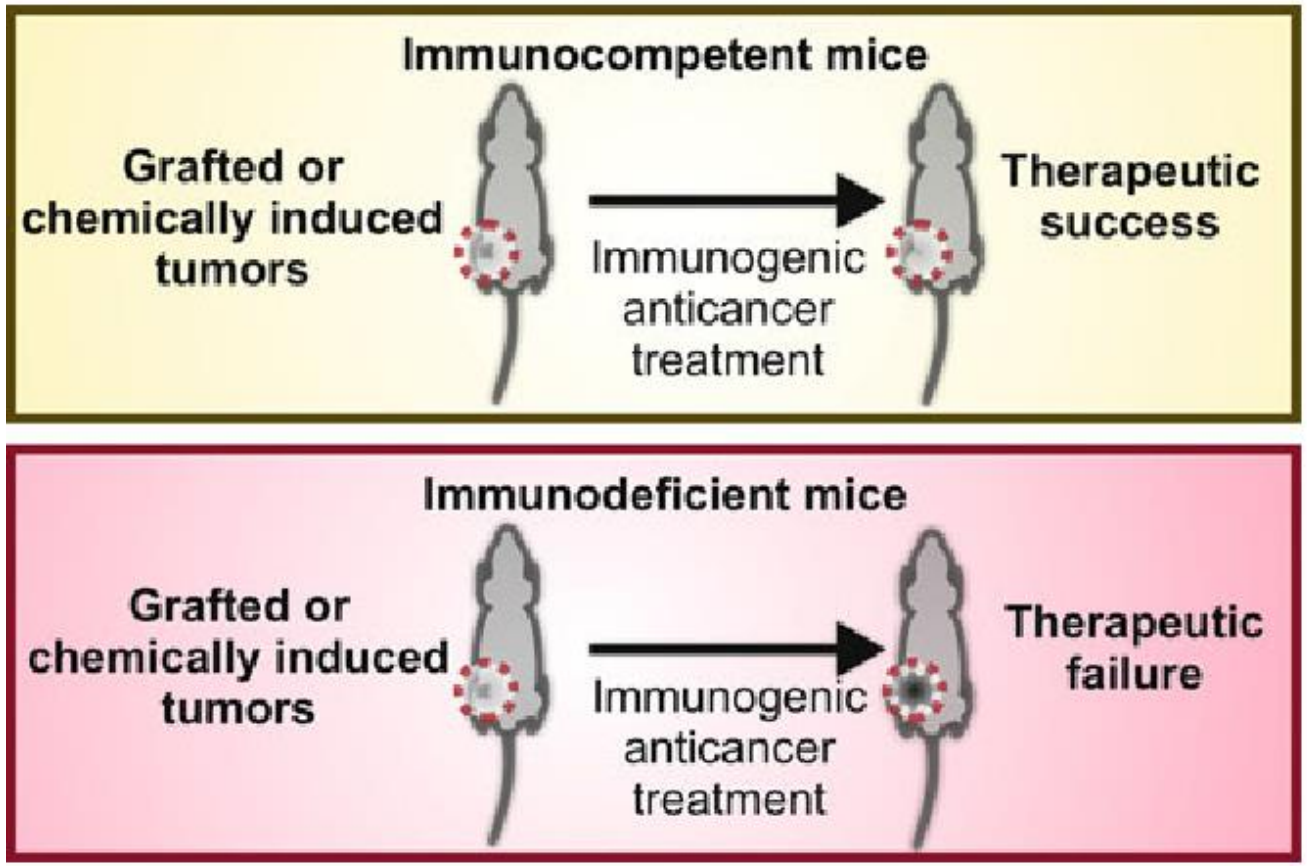


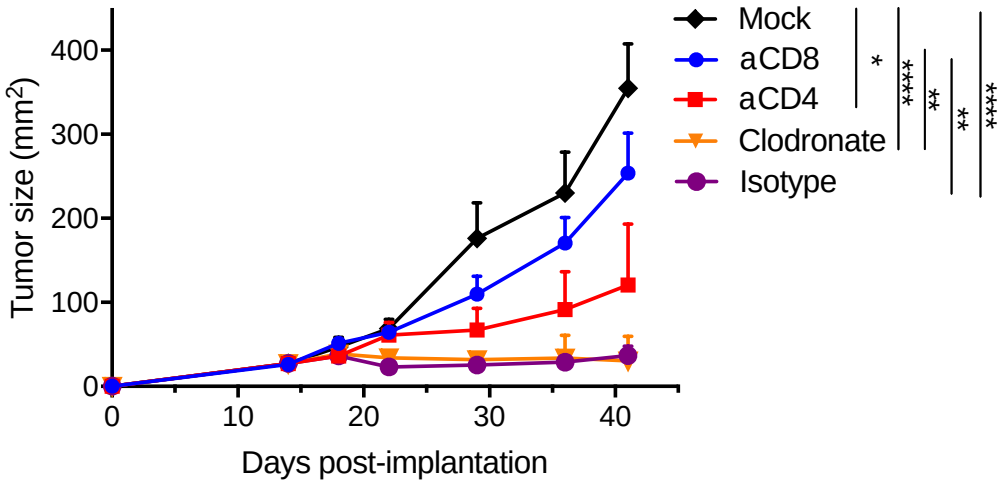


Ecto-calreticulin (green)

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

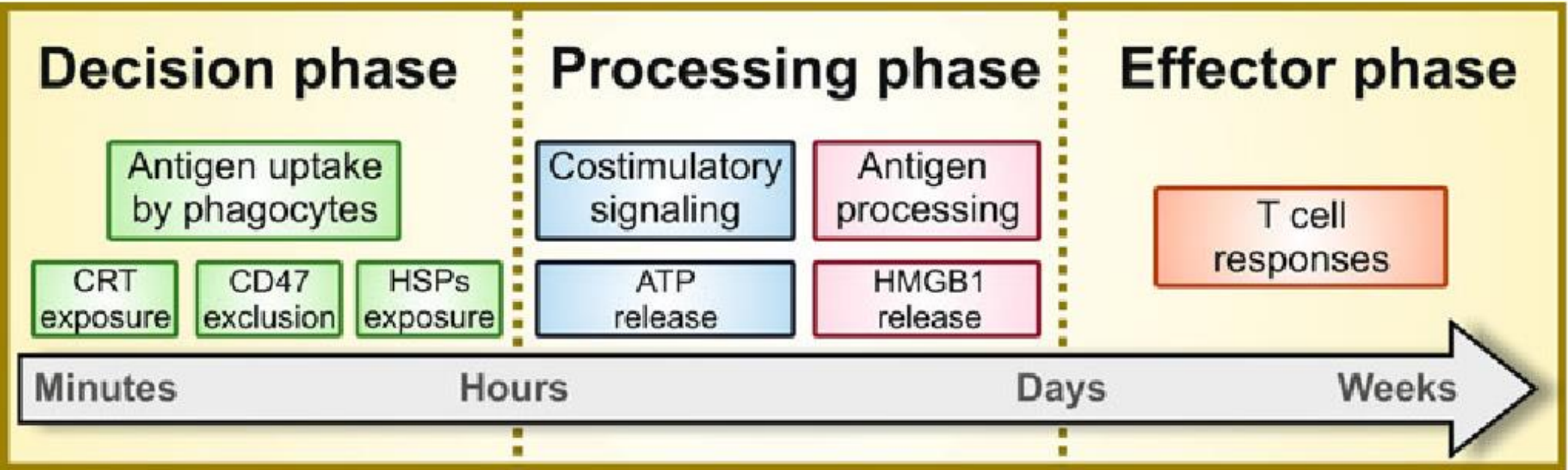
Contemporary definition: Immune induction





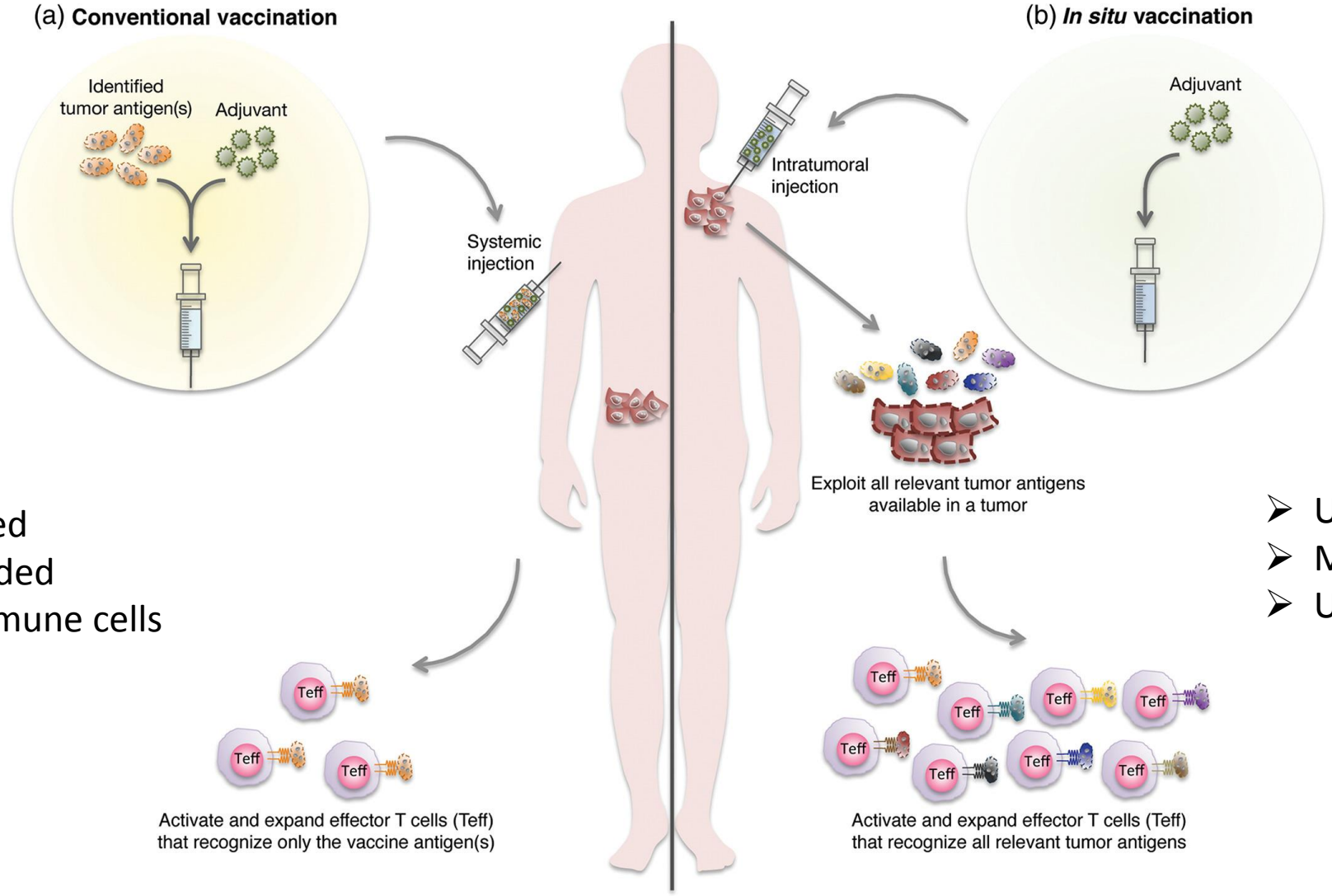
Kepp, Galluzzi, et al. Cancer Metastasis Rev 2011
Bommareddy et al. Science Transl Med 2018

Spatiotemporal “sensing” of ICD by the immune system



Kepp, Galluzzi, et al. Cancer Metastasis Rev 2011

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

Intratumoral Immunotherapy

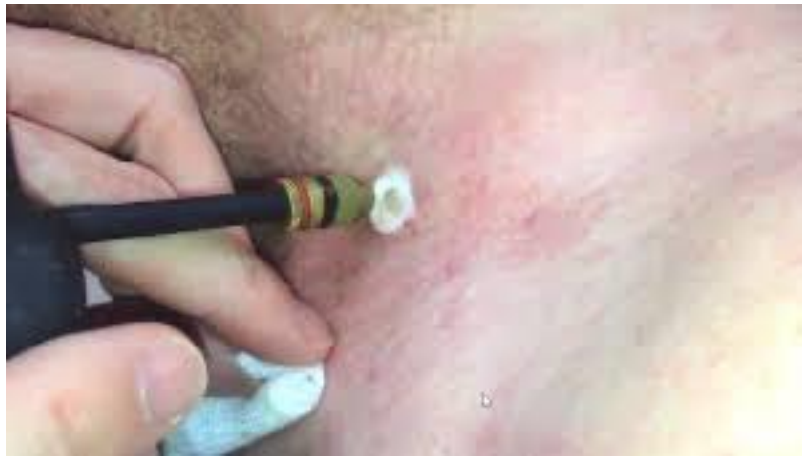
Types of Intratumoral Therapy

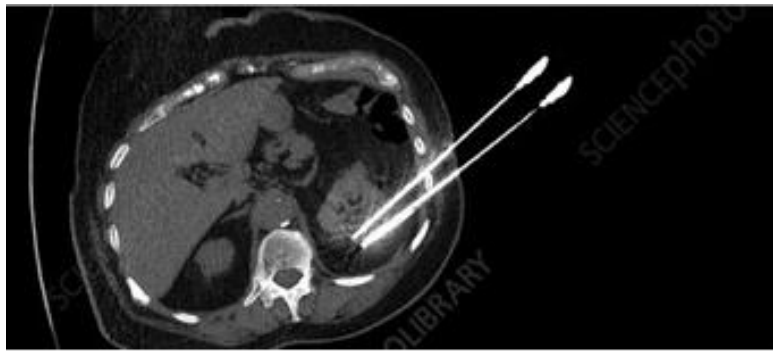
Types of Intra-tumoral therapy

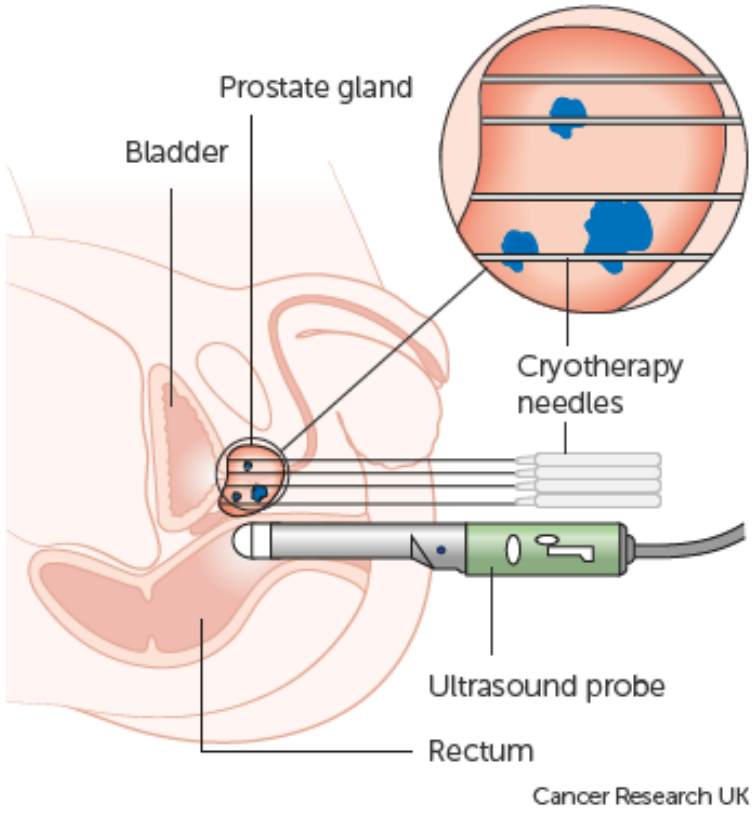
- Physical (Ablative) therapies
  - Cryotherapy
  - Microwave and Radiofrequency ablation
  - Focused ultrasound
  - Hyperthermia
  - Radiation
  - Electroporation
- Drug-related therapies
  - Oncolytic viruses
  - Direct Delivery of Anti-neoplastic Agents
  - Intraumoral cytokines
  - Intratumoral immune checkpoint inhibitor mAbs
  - Intratumoral immune agonists (TLR, cGAS-STING)
  - Intratumoral cell therapy (DC, T cells, etc.)
  - Intratumoral chemotherapy
- Combination therapy
  - Intratumoral and intratumoral
  - Intratumoral and systemic

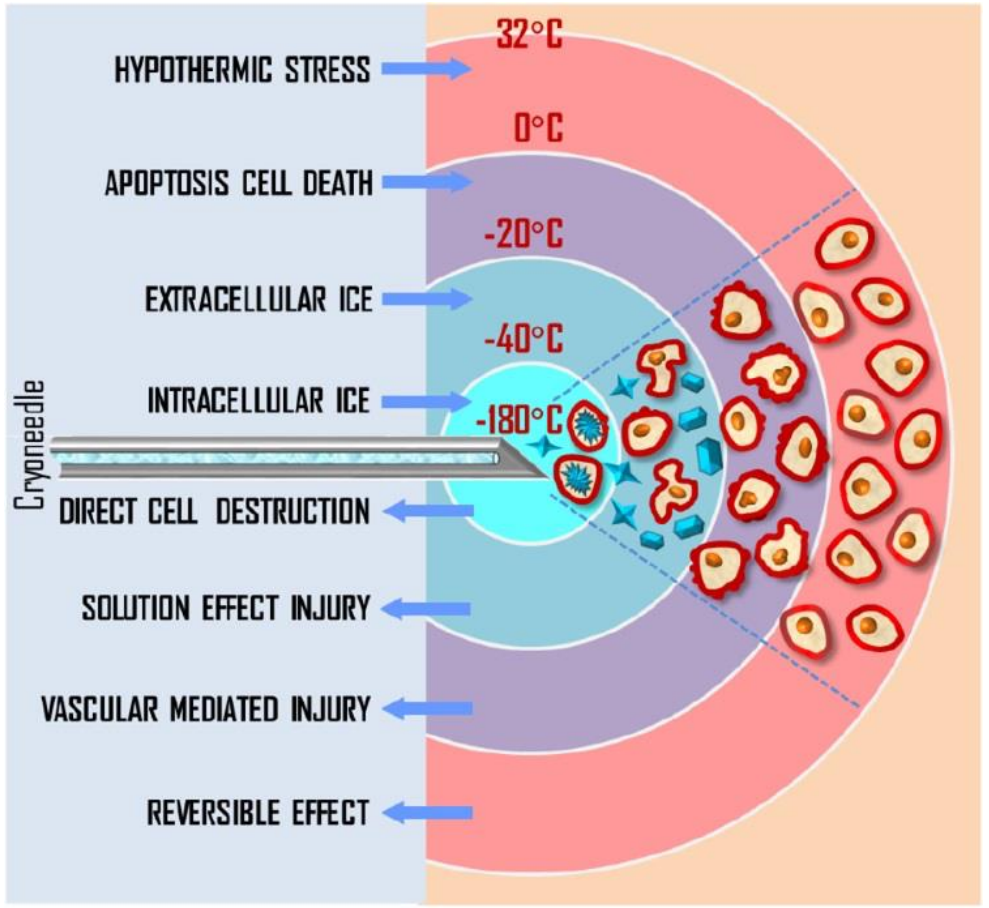
Physical Intratumoral Therapy

Cryotherapy





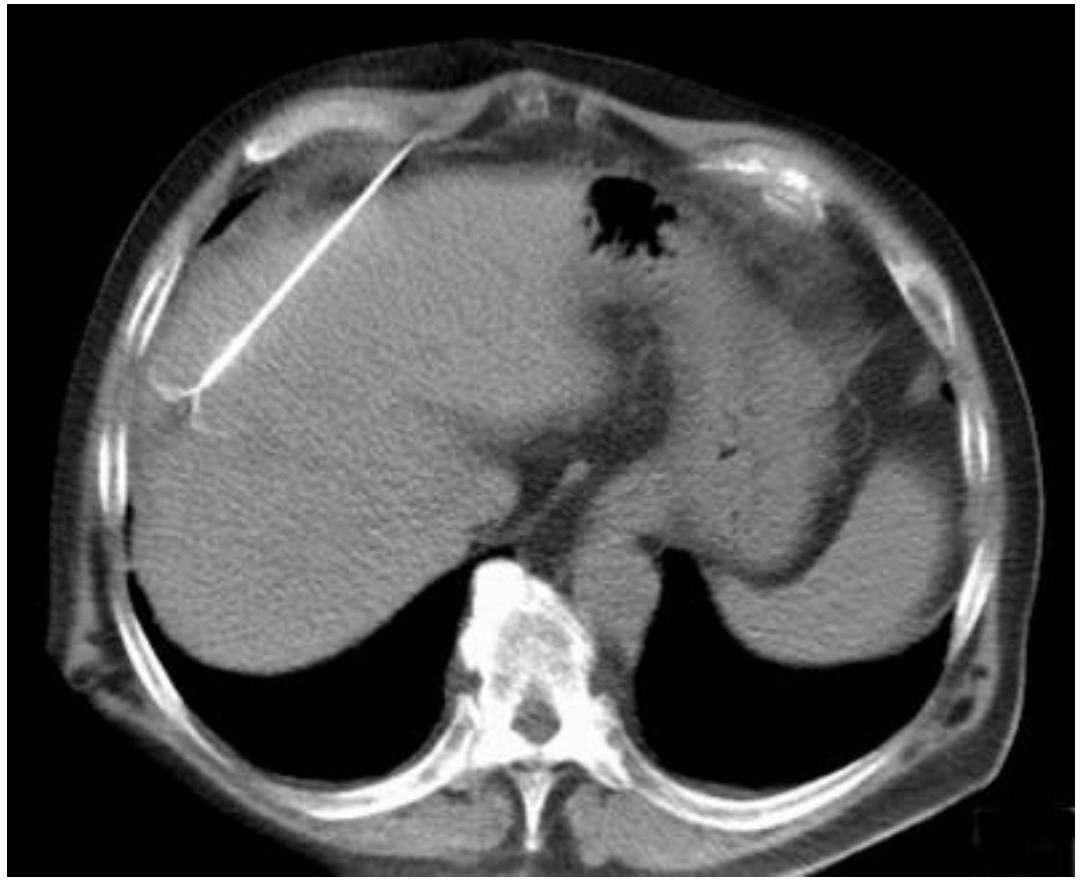


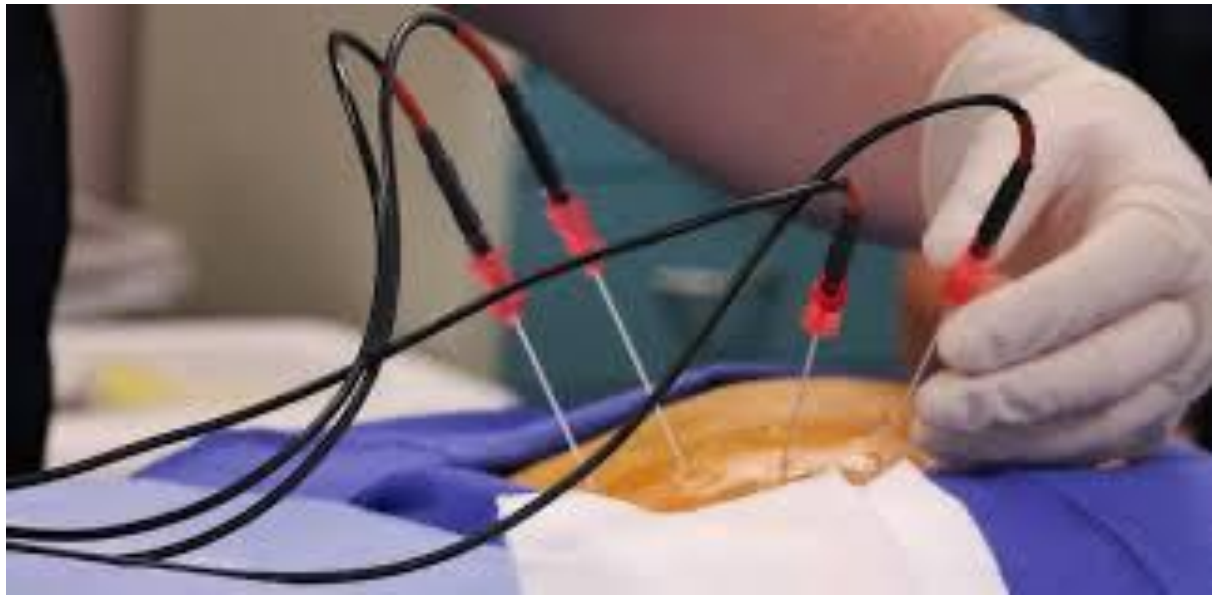


Toxicity:

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

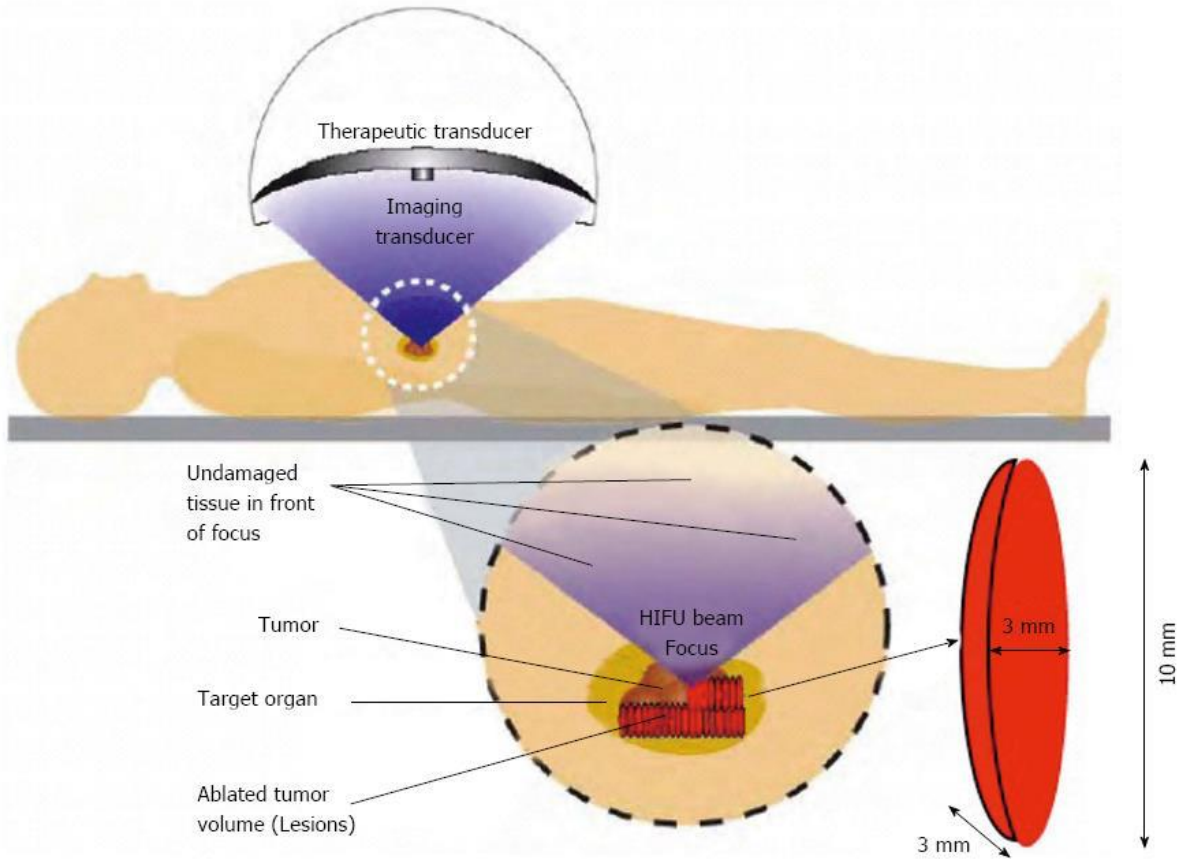
Microwave and Radiofrequency Ablation





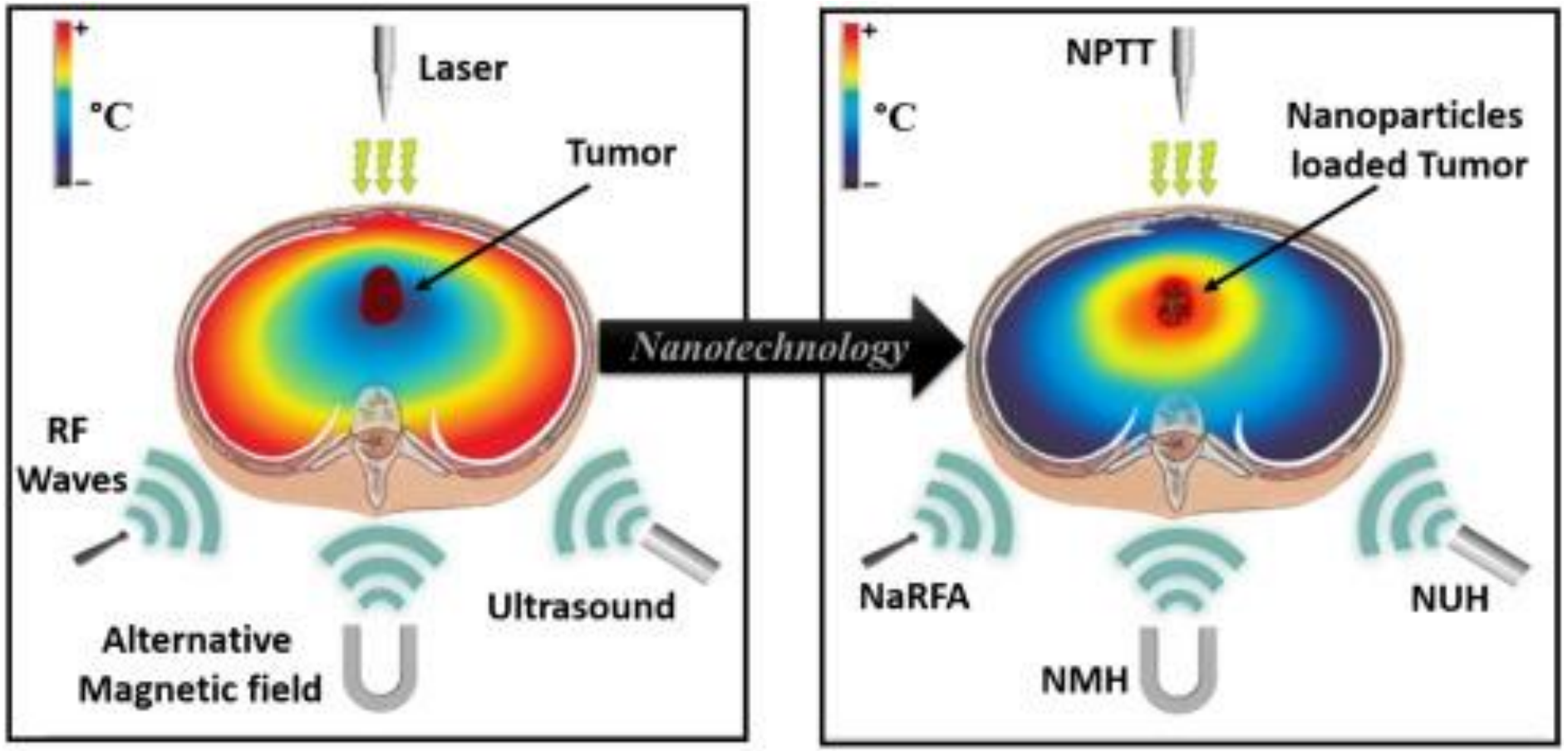
- Tumor entered with thin needle and probe
- Apply electrical current (radiofrequency) or microwave energy
- Tumor necrosis induced
- Residual scar left behind

High-intensity Focused Ultrasound



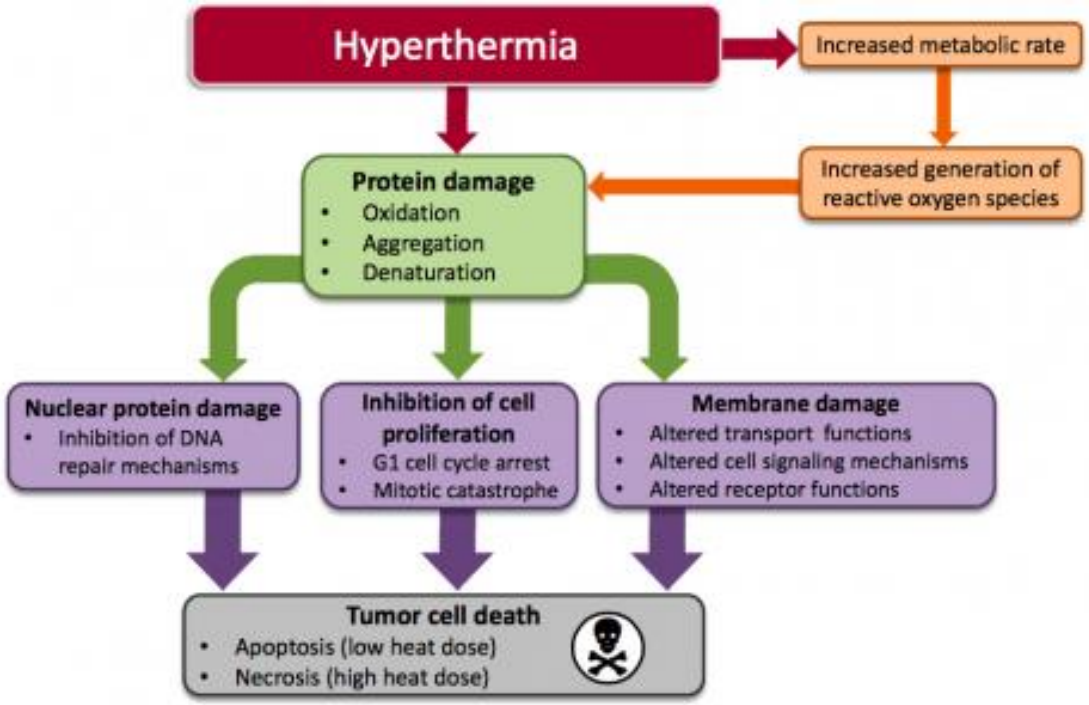
- Non-invasive therapeutic technique
- Uses lower frequency and continuous waves
- Induces thermal damage in tissue (65-85 °C)
- Pulsed waves induce mechanical damage
- Can use with ultrasound or MRI imaging
- HIFU approved in U.S. for prostate cancer treatment in 2015
- Many other tumors under study

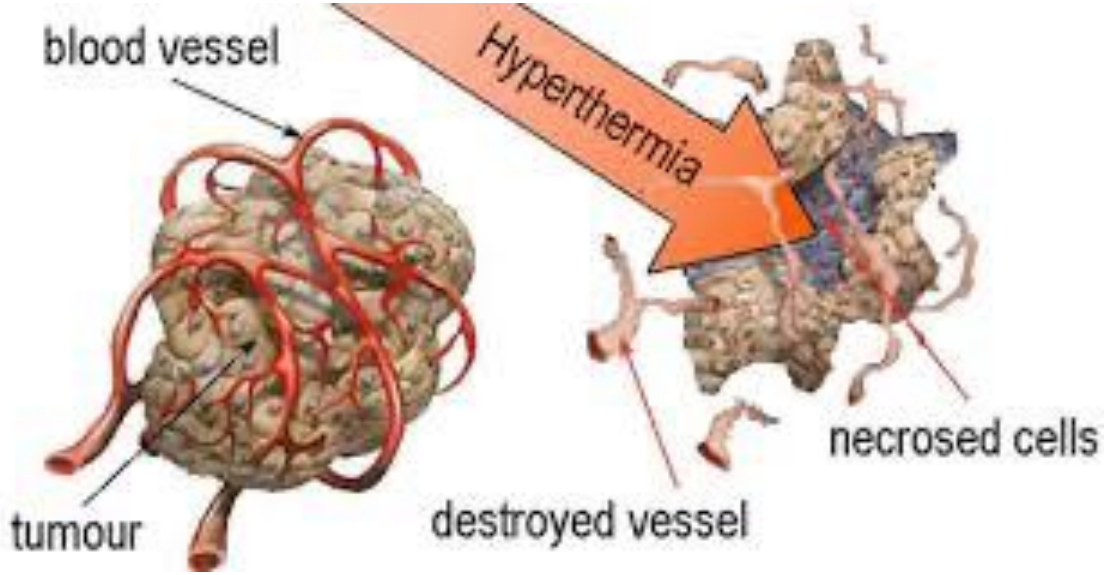
Hyperthermia



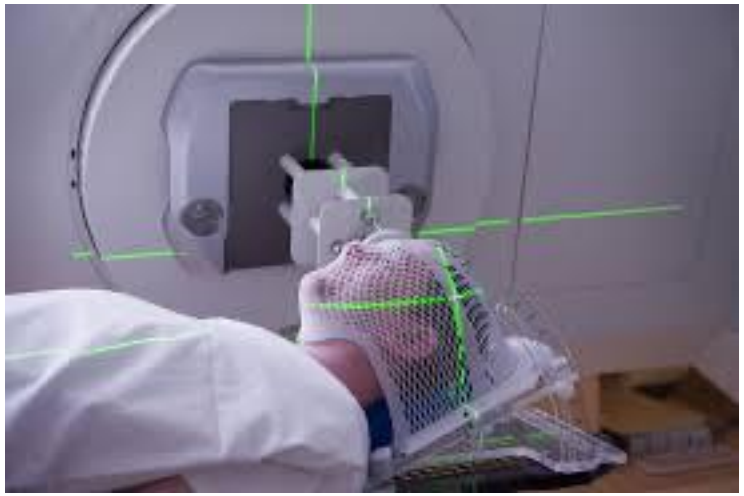
Beik et al. J Controlled Rel 2016

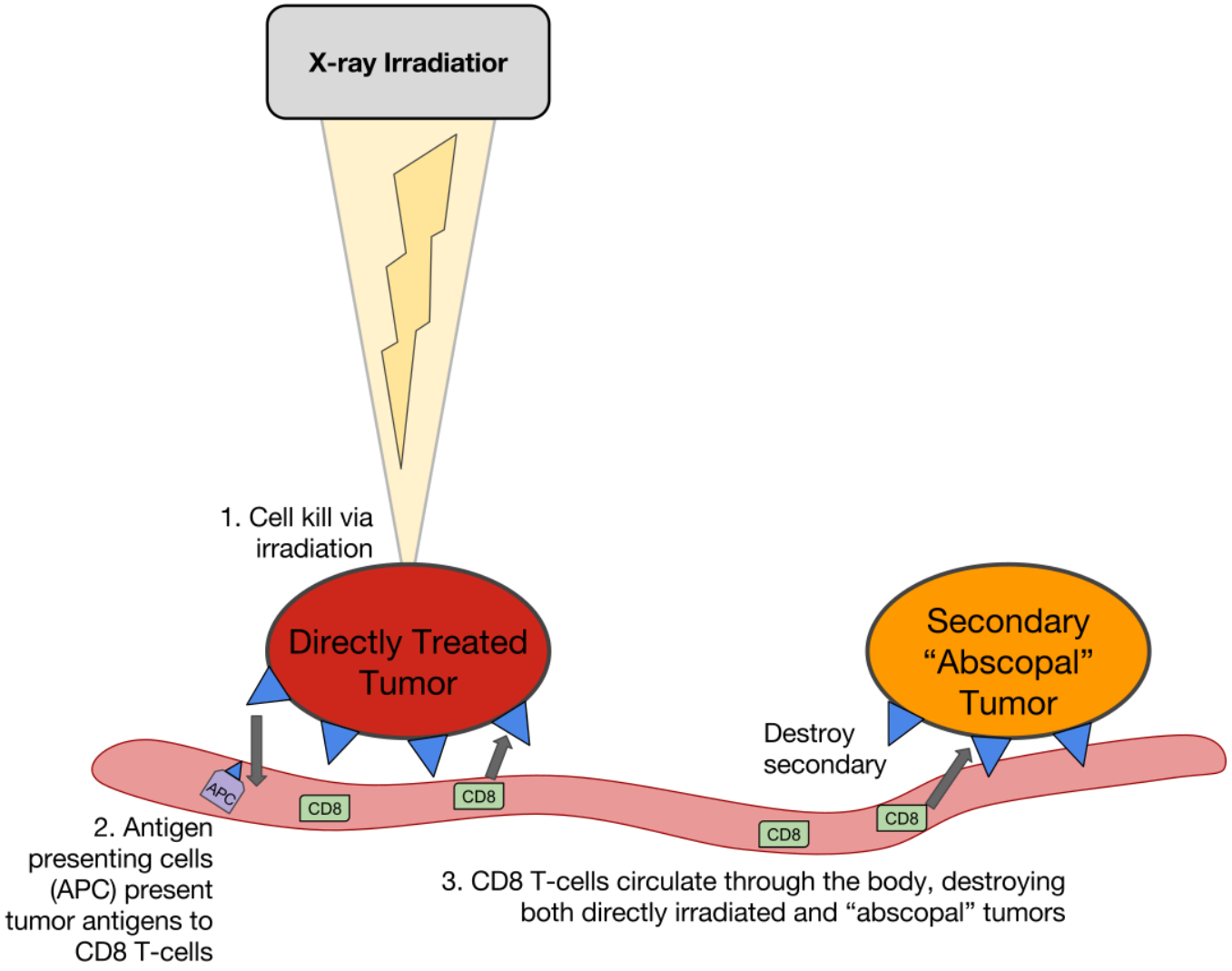
How does hyperthermia mediate anti-tumor activity?



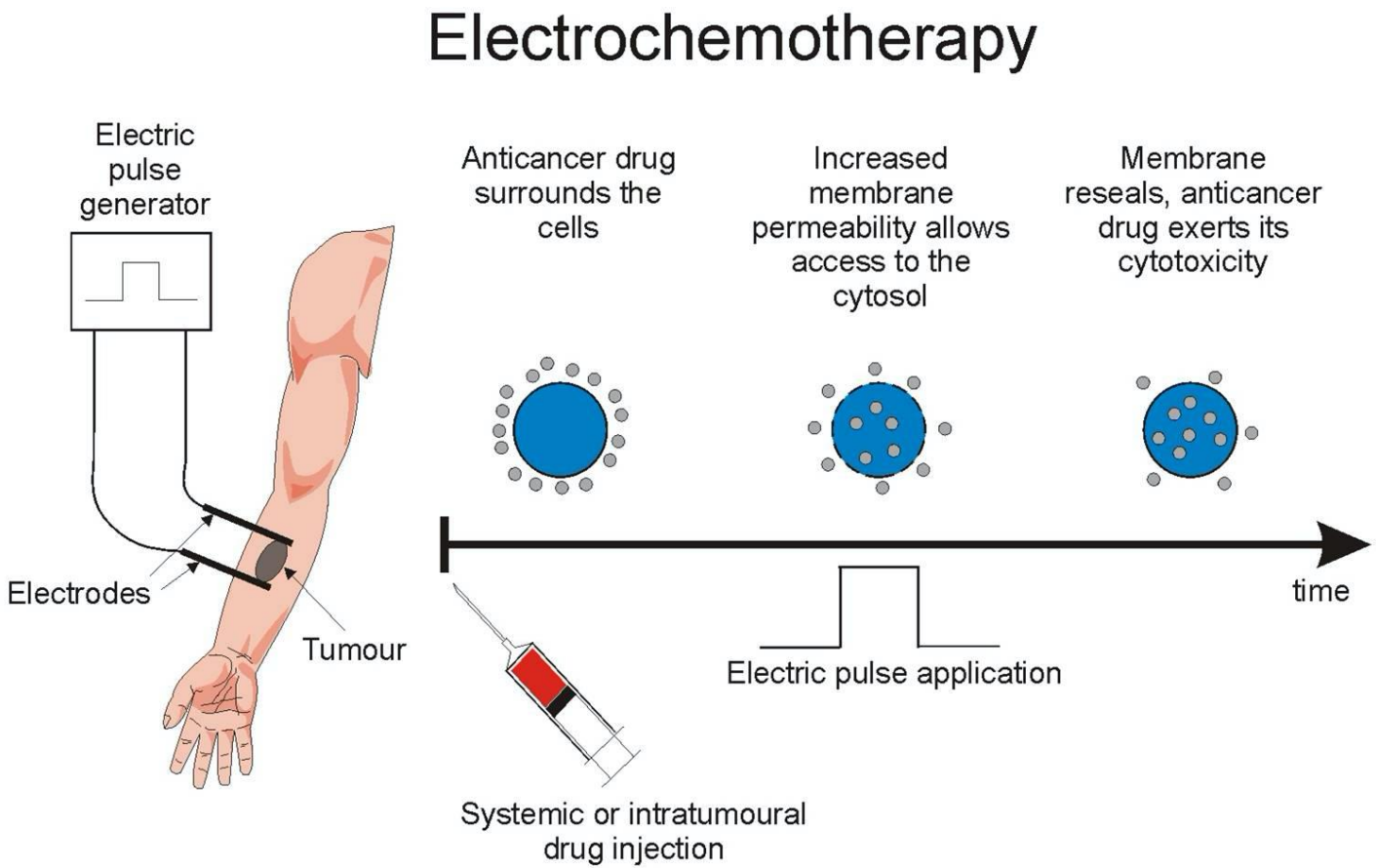


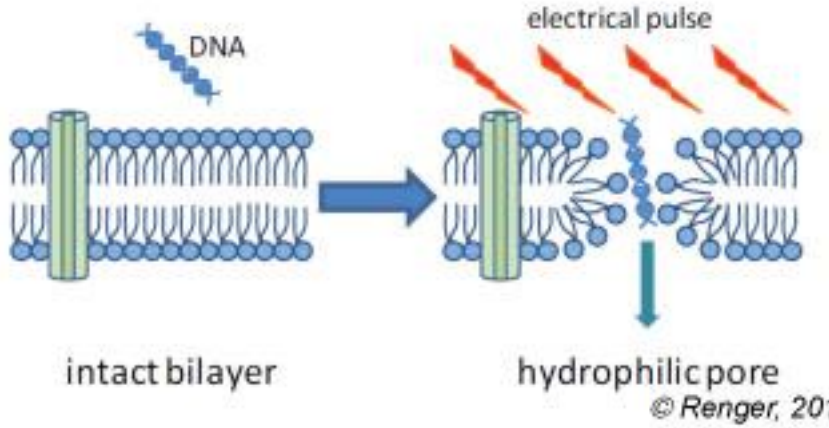
Radiation Therapy

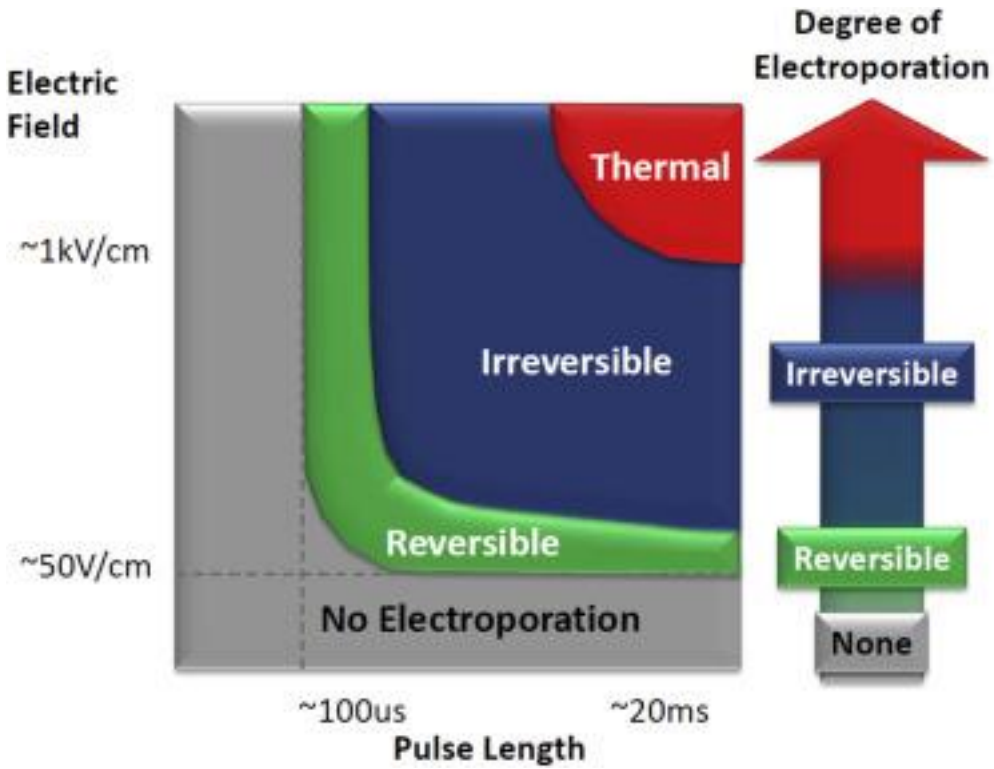




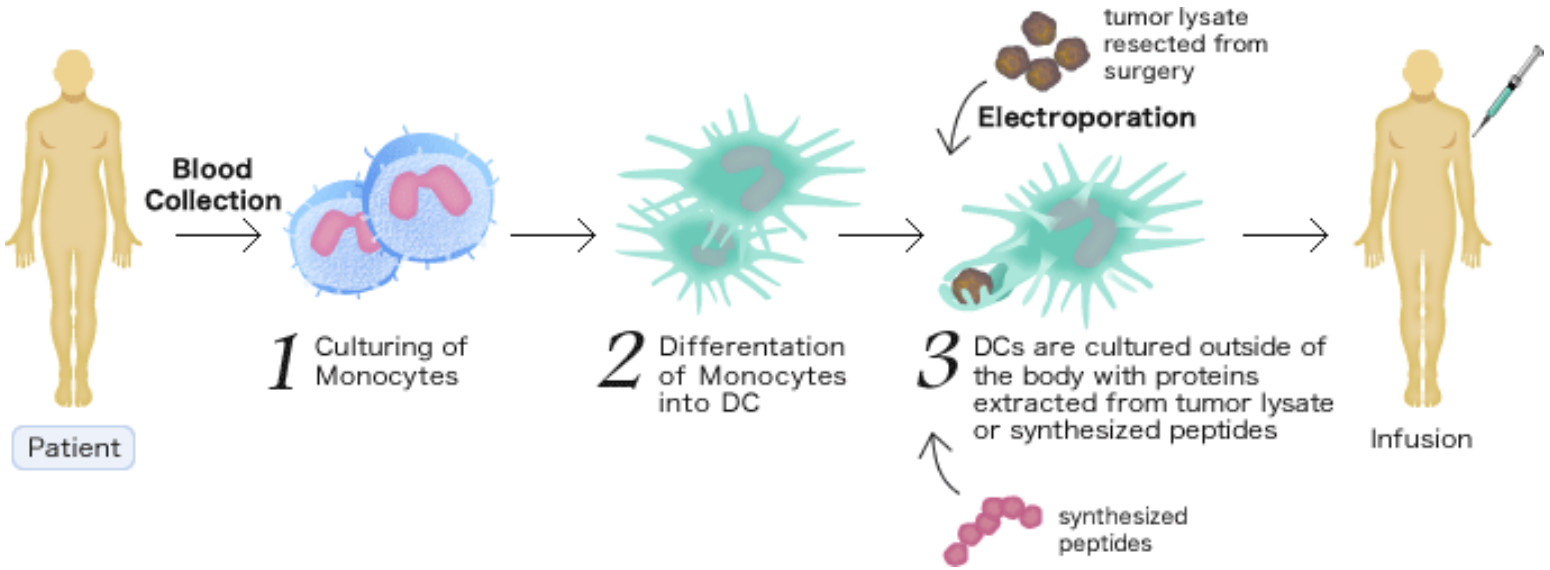
Electroporation





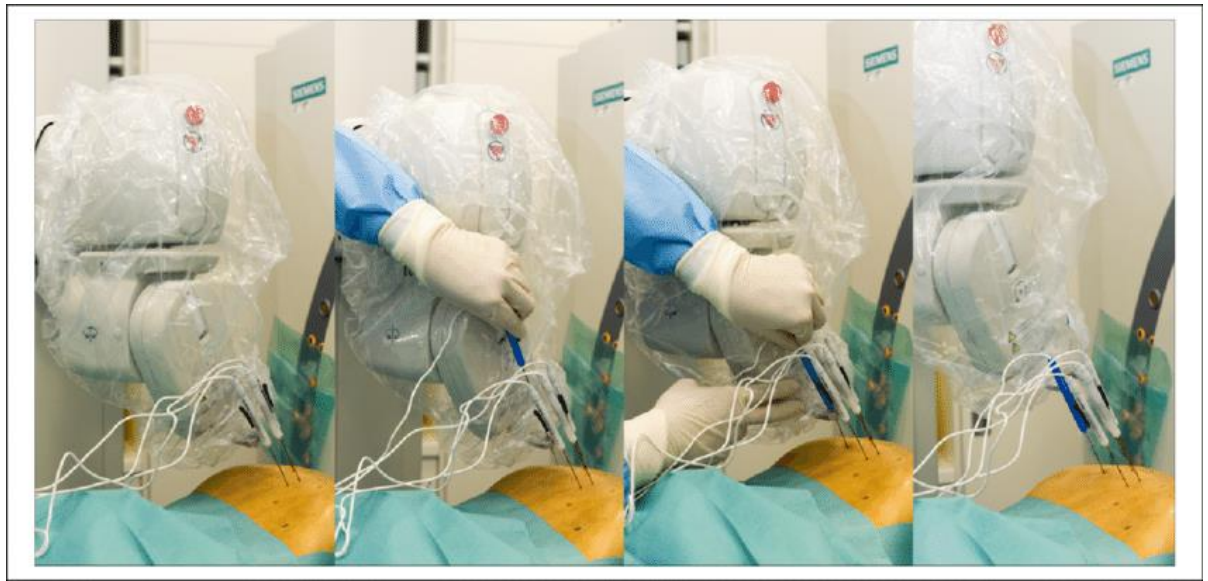


Types of Electroporation



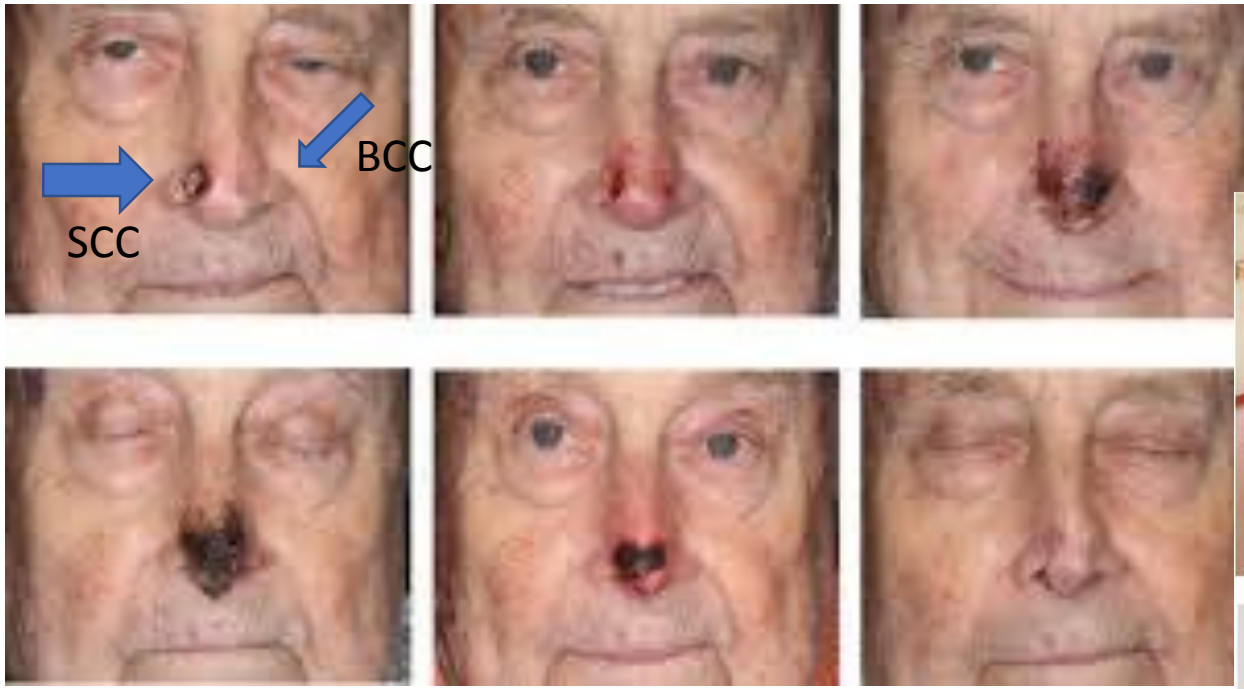
Ex vivo electroporation

Robot-assisted irreversible ***in vivo*** electroporation of hepatic metastases



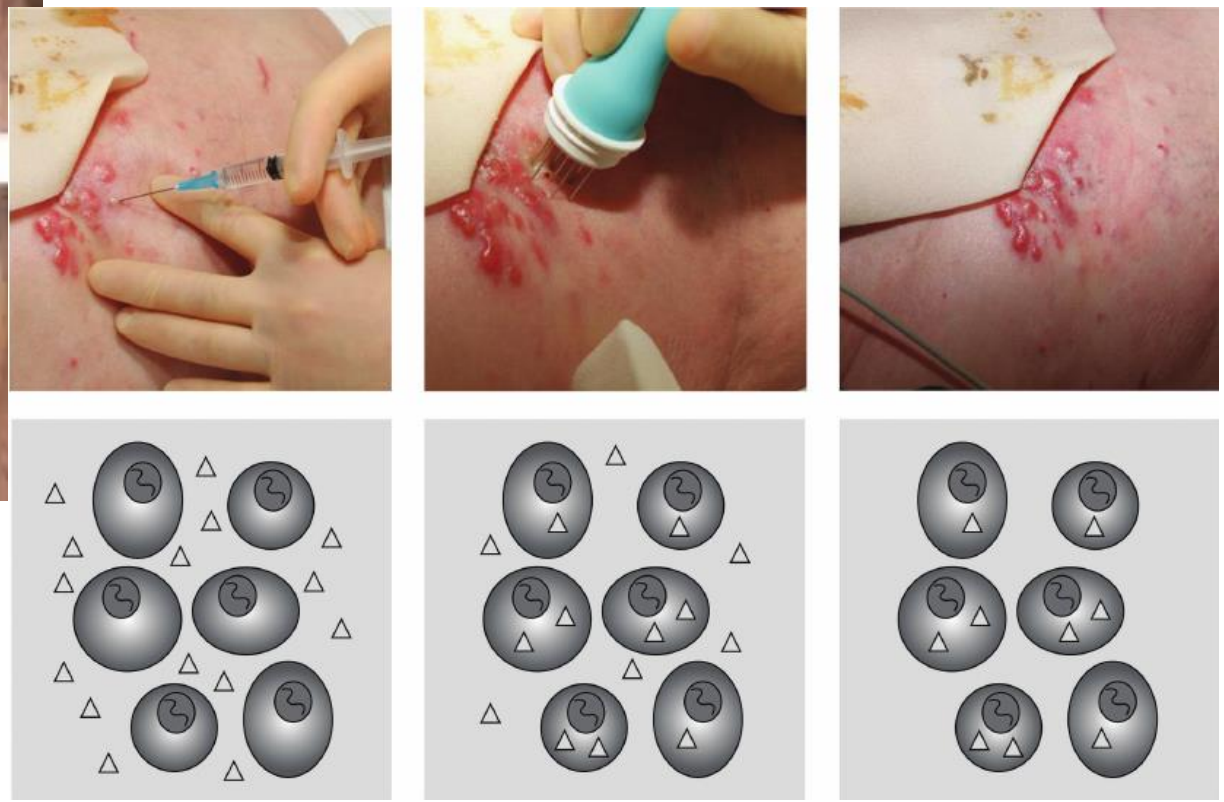
Drug-related Intratumoral Therapy

Intratumoral chemotherapy and electrochemotherapy



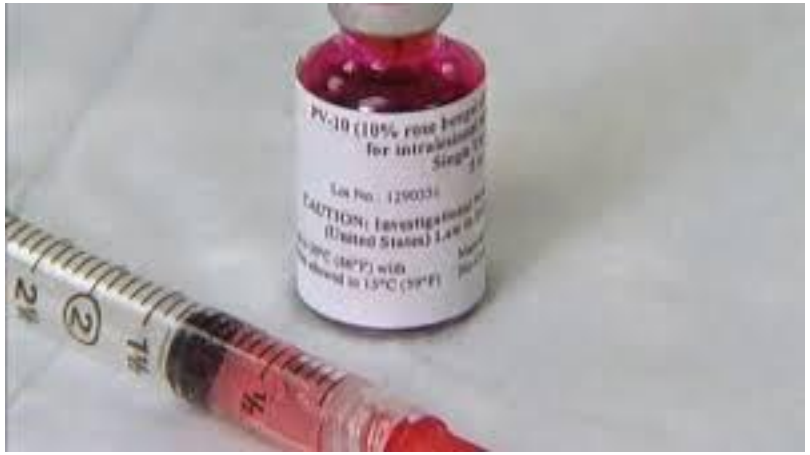
Treated with six weekly intra-lesional injections of 5-FU

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

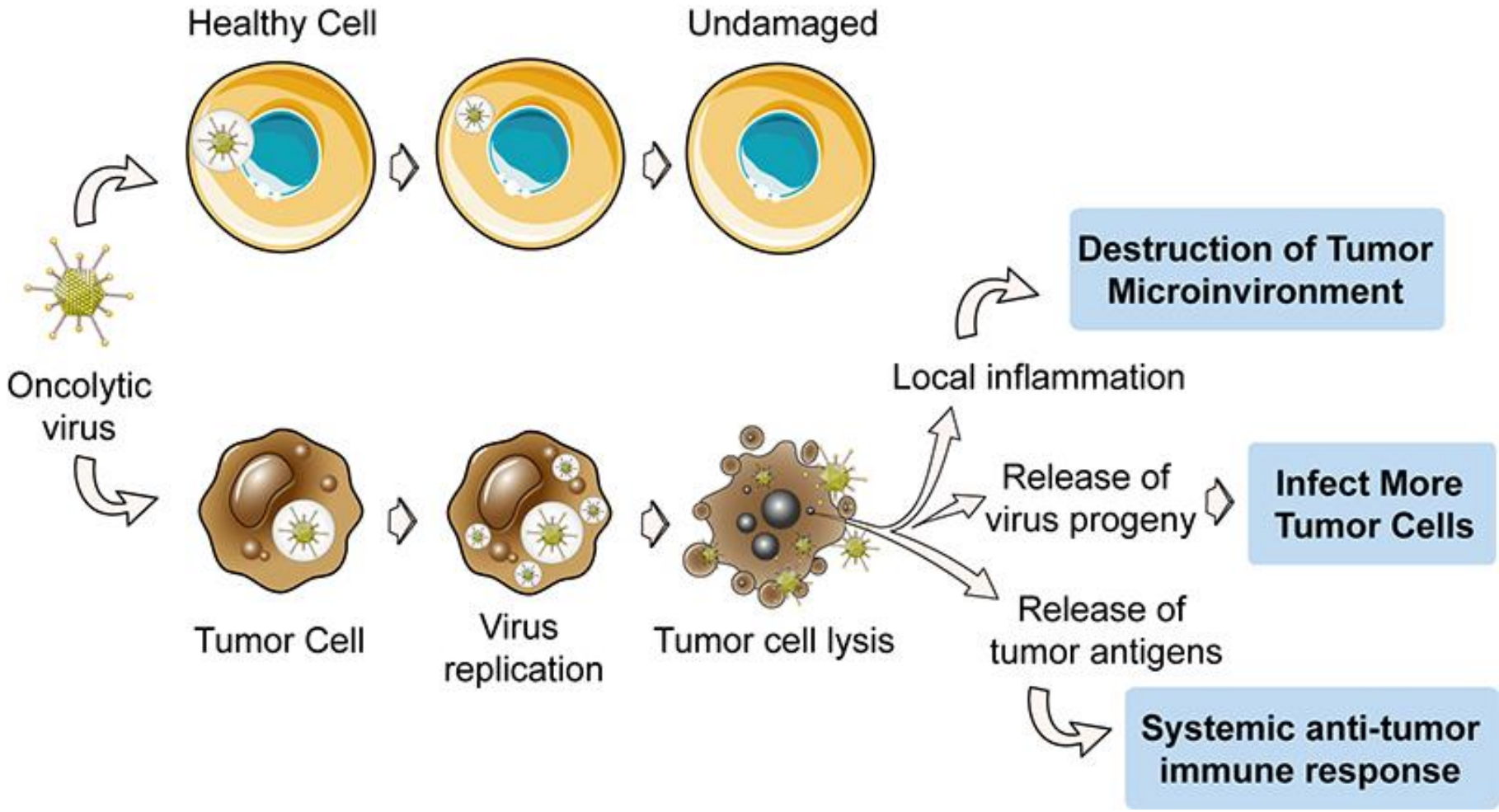
In-transit mets
45 patients

- 87% ORR
- 42% CR

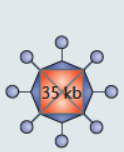
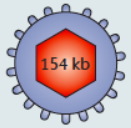
Read et al. J Surg Oncol 2018

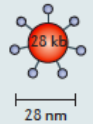
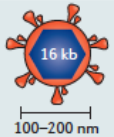
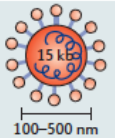
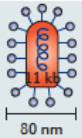
Oncolytic Viruses

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



Oncolytic Viruses

	Adenovirus	Vaccinia virus	Herpesvirus	Parvovirus H1
				
Baltimore classification	Group I: dsDNA	Group I: dsDNA	Group I: dsDNA	Group II: ssDNA
Family	Adenoviridae	Poxviridae	Herpesviridae	Parvoviridae
Virion	Naked	Complex coats	Enveloped	Naked
Capsid symmetry	Icosahedral	Complex	Icosahedral	Icosahedral
Replication site	Nucleus and cytoplasm	Cytoplasm	Nucleus and cytoplasm	Nucleus and cytoplasm
Cell receptor	CAR	Unknown	HVEM, nectin 1, nectin 2	Sialic acid residues
Nuclear integration	+	+	+	+
Transgene capacity	++	+++	+++	N/A
Wild-type virus infects non-replicating cells	–	–	–	+
Virulence of wild-type virus	+/–	+/–	–	+
Antivirals	+	+	+	–
Immunogenicity	–	–	–	+
Haemagglutination	–	–	–	+
Blood–brain barrier penetration	–	–	–	+
Achievable titre (PFU per ml)	10^{12}	10^{9}	10^{10}	5×10^{8}
MTD	3×10^{12}	3×10^{9}	10^{9}	N/A

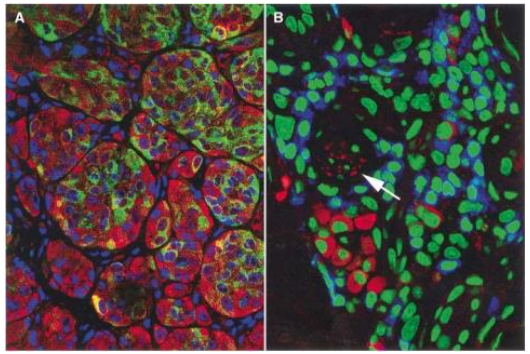
	Reovirus	Coxsackievirus	Seneca Valley Virus	Poliovirus	Measles virus	Newcastle disease virus	Vesicular stomatitis virus
							
Baltimore classification	Group III: dsRNA	Group IV: ssRNA	Group IV: ss(+) RNA	Group IV: ss(+) RNA	Group V: ss(–) RNA	Group V: ss(–) RNA	Group V ss(–) RNA
Family	Reoviridae	Picornaviridae	Picornaviridae	Picornaviridae	Paramyxoviridae	Paramyxoviridae	Rhabdoviridae
Virion	Naked	Naked	Naked	Naked	Enveloped	Enveloped	Enveloped
Capsid symmetry	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Helical	Helical
Replication site	Cytoplasm	Cytoplasm	Cytoplasm	Cytoplasm	Cytoplasm	Cytoplasm	Cytoplasm
Cell receptor	Unknown	CAR/ICAM-1/ DAF	Unknown	CD155	SLAM and CD46	Unknown	LDLR
Nuclear integration	+	+	+	+	+	+	+
Transgene capacity	N/A	N/A	N/A	N/A	+	+	+
Wild-type virus infects non-replicating cells	+	–	+	–	–	–	+
Virulence of wild-type virus	+	+/–	+	–	–	+	+
Antivirals	–	–	–	–	–	–	–
Immunogenicity	–	–	+	+/–	–	–	–
Haemagglutination	–	+	+	+	–	–	–
Blood–brain barrier penetration	+	–	+	+	–	+	–
Achievable titre (PFU per ml)	10^{9}	10^{9}	N/A	10^{8}	10^{11}	10^{8}	2×10^{10}
MTD	3×10^{10}	10^{9}	10^{11} VP per kg	NA	10^{9}	Initial 10^{9}; subsequent 10^{10}	N/A

Kaufman et al. Nature Rev DD 2015

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



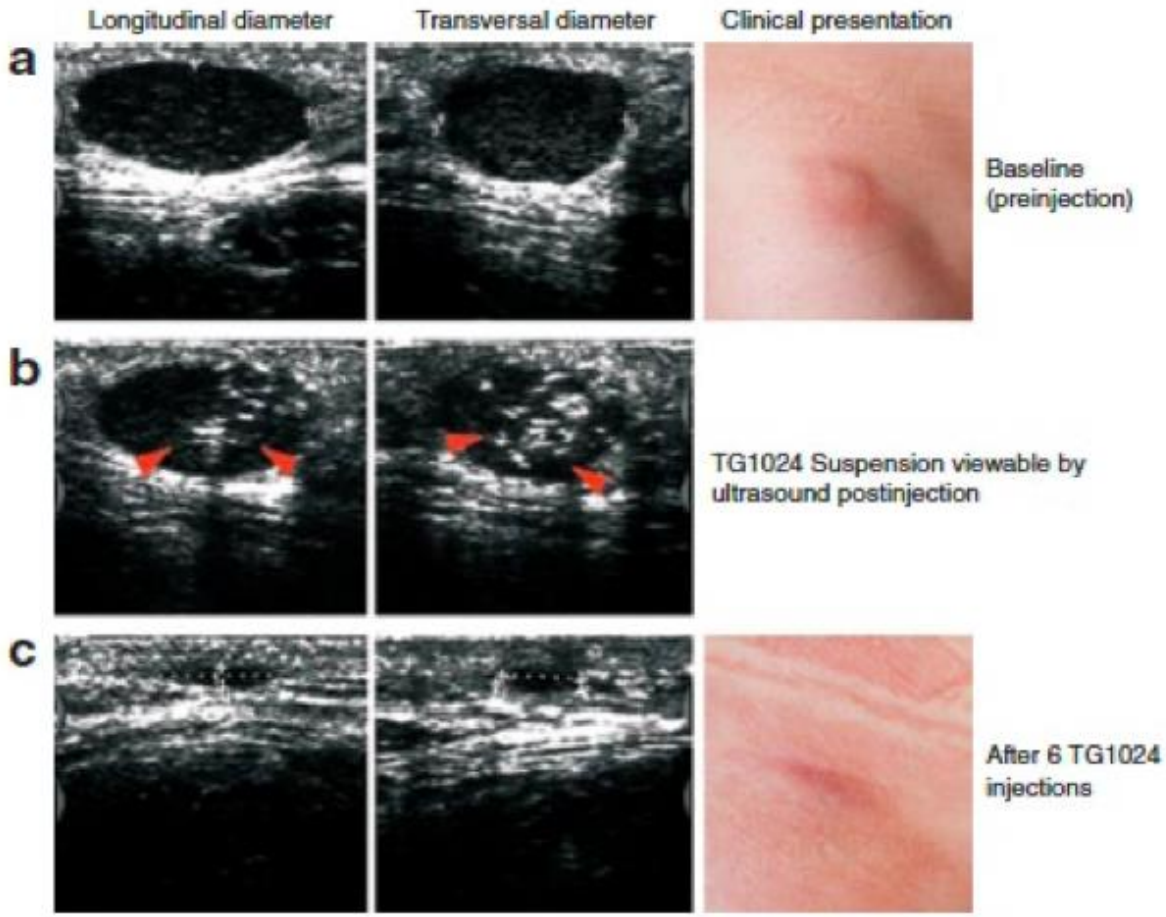
Meta-analysis of 49 studies of intra-lesional IL-2 for in-transit melanoma

- 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

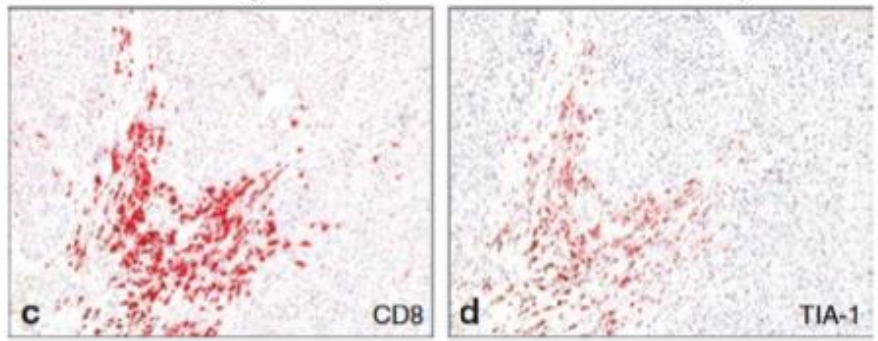
Intralesional Adenovirus-Mediated IL-2 Gene Transfer for Advanced Solid Cancers and Melanoma

Clinical Response of an Injected Tumor



17% (6/35) of patients had intralesional responses, 4 with concomitant stable disease (SD) in noninjected lesions

Inflammatory Response After 2 Injections



Dummer R, et al. *Mol Ther.* 2008;16(5):985-994.

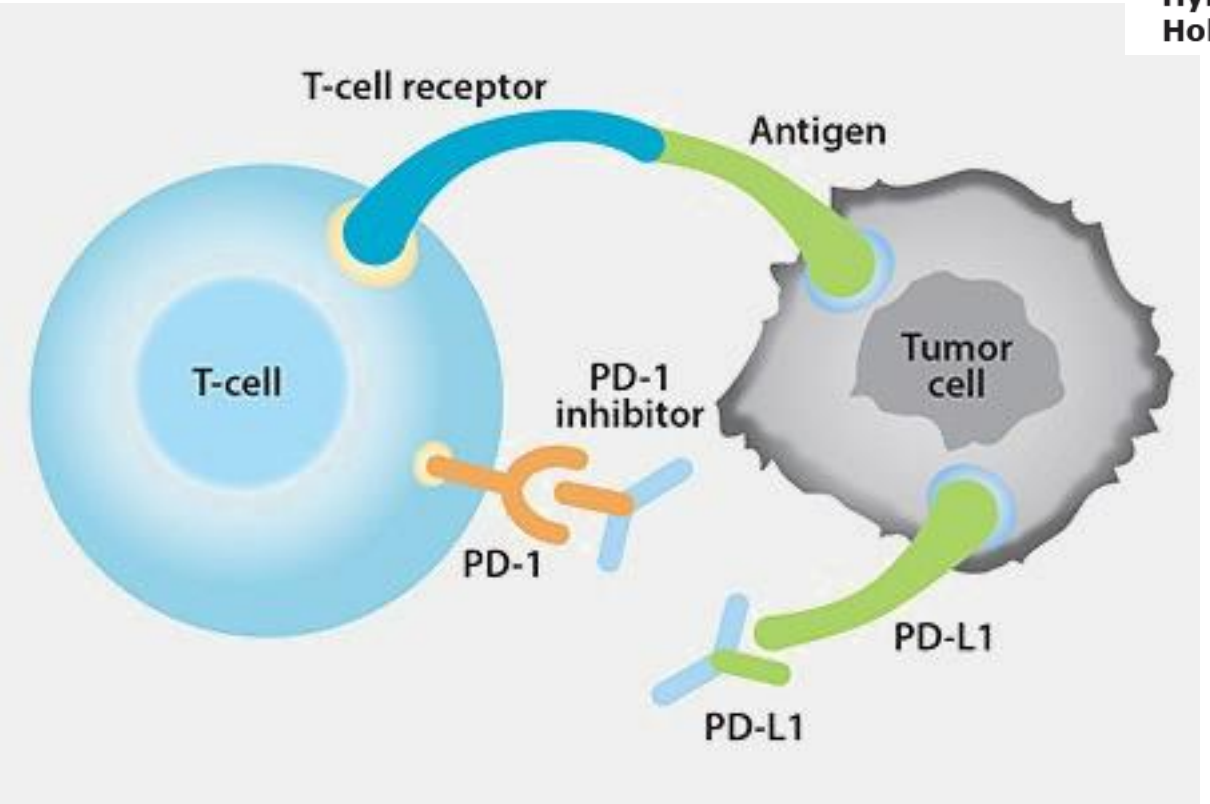
Intratumoral immune checkpoint inhibitor mAbs

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

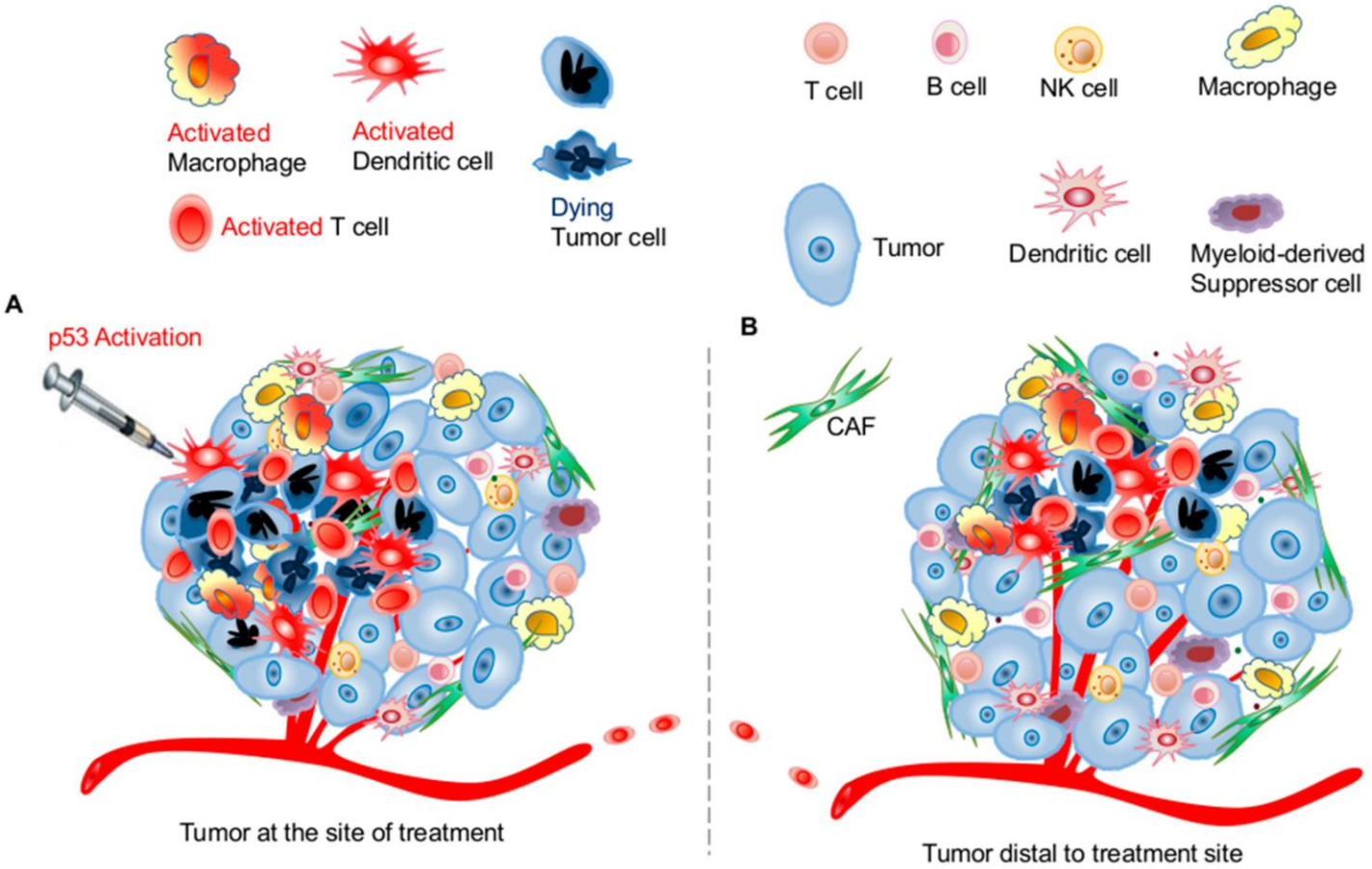
Abhijit Ray[1,*], Matthew A. Williams[2,*], Stephanie M. Meek[2], Randy C. Bowen[3], Kenneth F. Grossmann[1], Robert H.I. Andtbacka[4], Tawnya L. Bowles[5], John R. Hyngstrom[4,5], Sancy A. Leachman[6], Douglas Grossman[1], Glen M. Bowen[1], Sheri L. Holmen[1], Matthew W. VanBrocklin[1], Gita Suneja[7]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

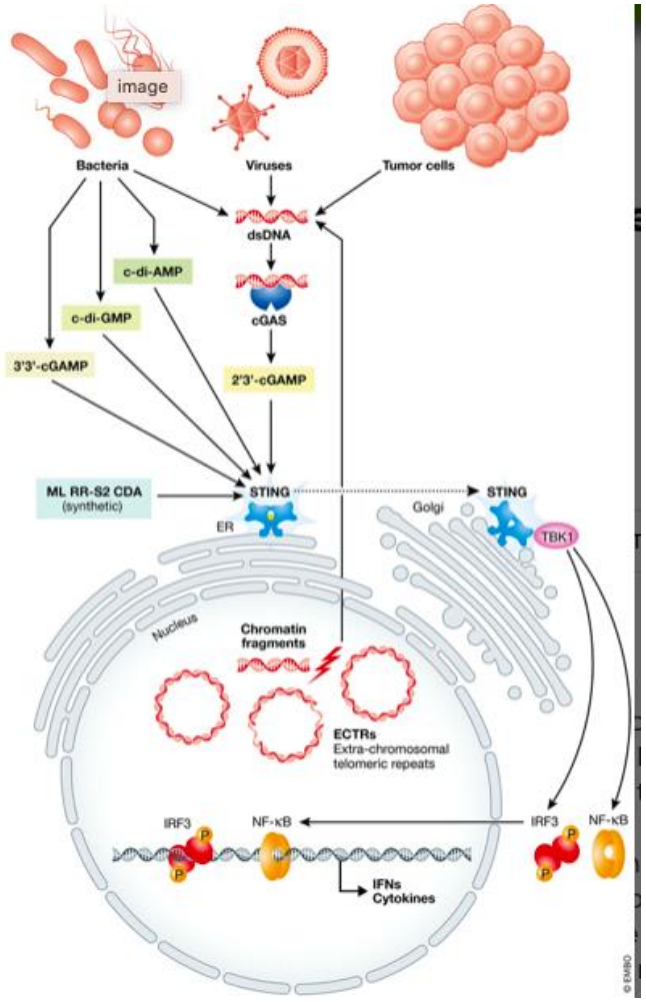


- Ex vivo modified cells
- In vivo modified cells

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

Cui and Guo Int J Mol Sci 2016

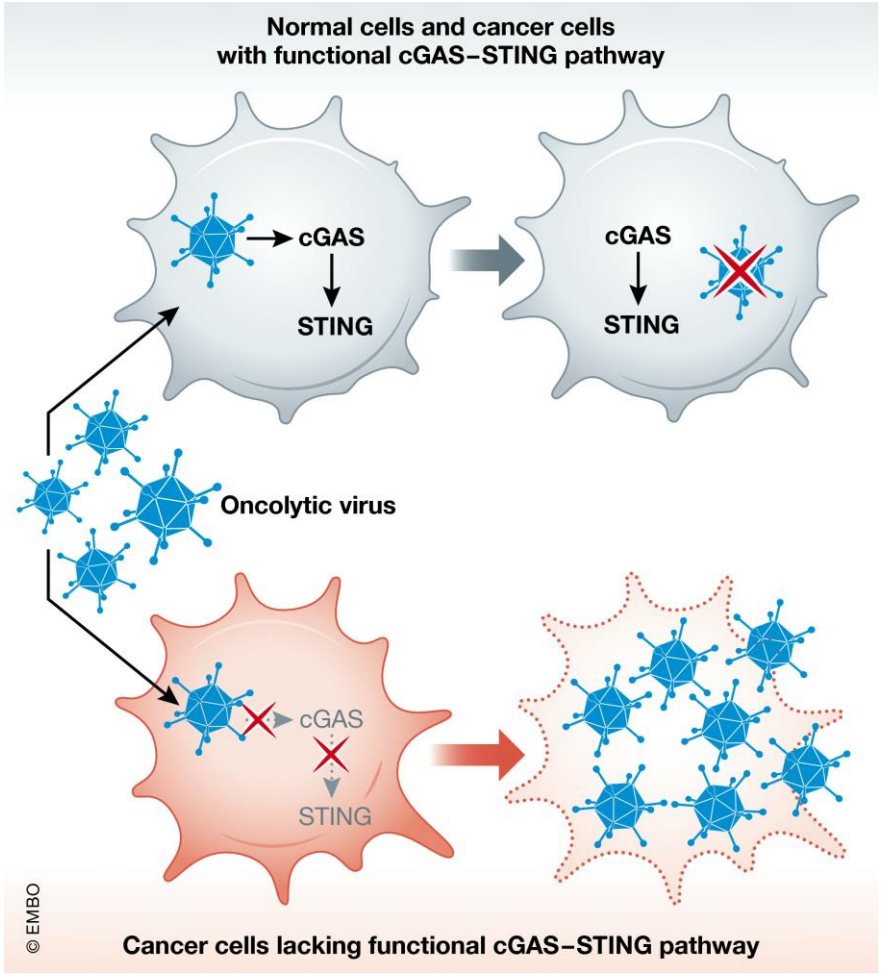
Intratumoral STING immune agonists



- 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

STING and oncolytic viruses



Decreased viral pathogenicity
Increased viral clearance
Protects normal cells from viral killing

Increased tumor cell killing
Decreased viral clearance
May increase viral immunogenicity

Khoo and Chen EMBO Rep 2018

Toll-like receptor agonists

Danger is represented by:

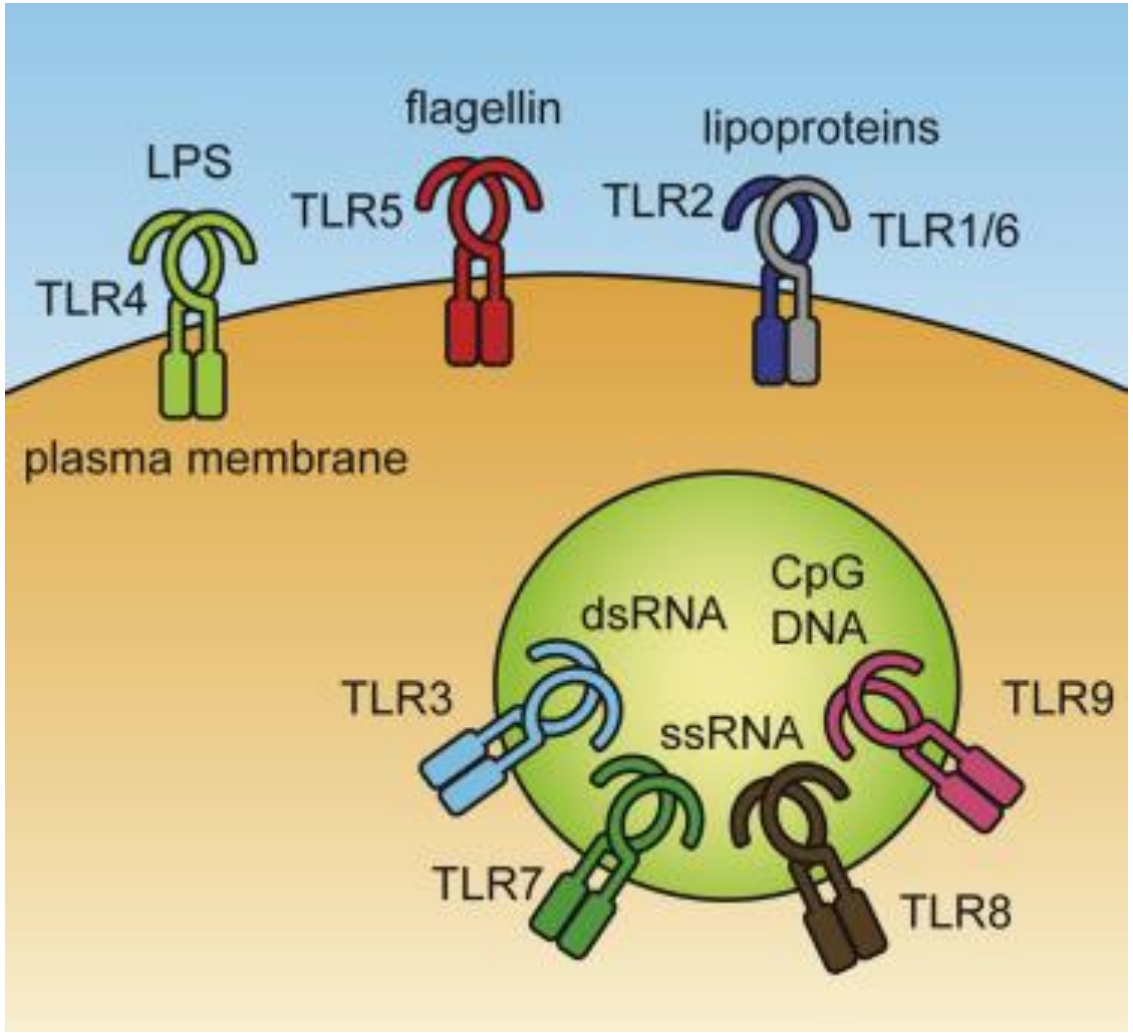
These have molecular features that distinguish them from our own cells:

endotoxin
DS RNA
HMGB1
lipopeptides
CpG DNA
SS RNA
flagellin

binds RAGE, LPS;
amplifies TLR sig

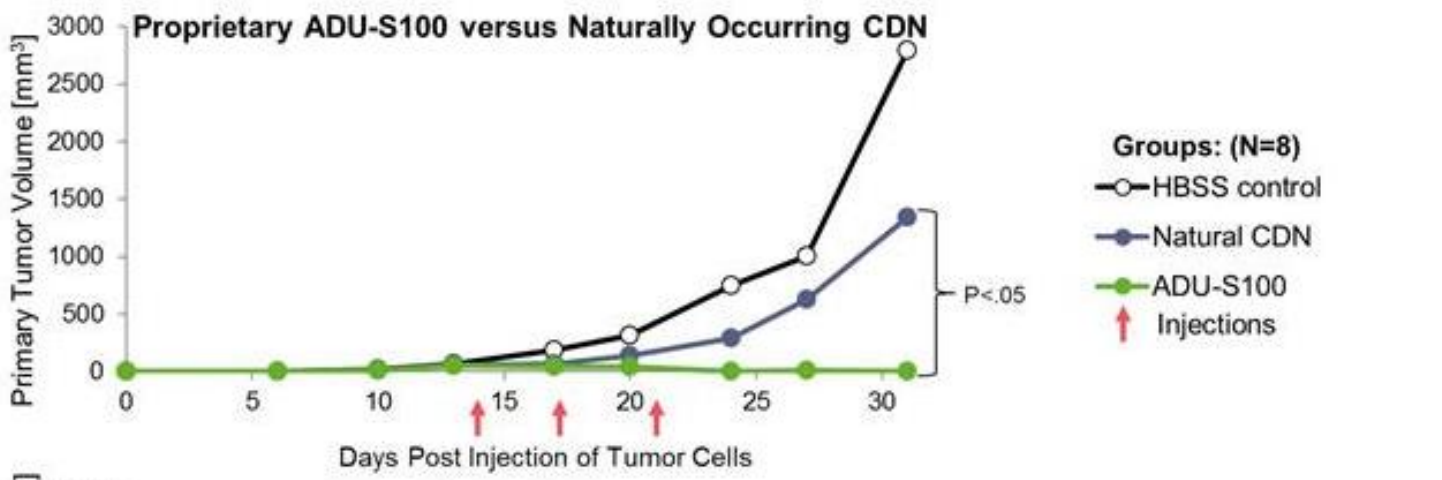
Our immune systems have evolved to recognize them:

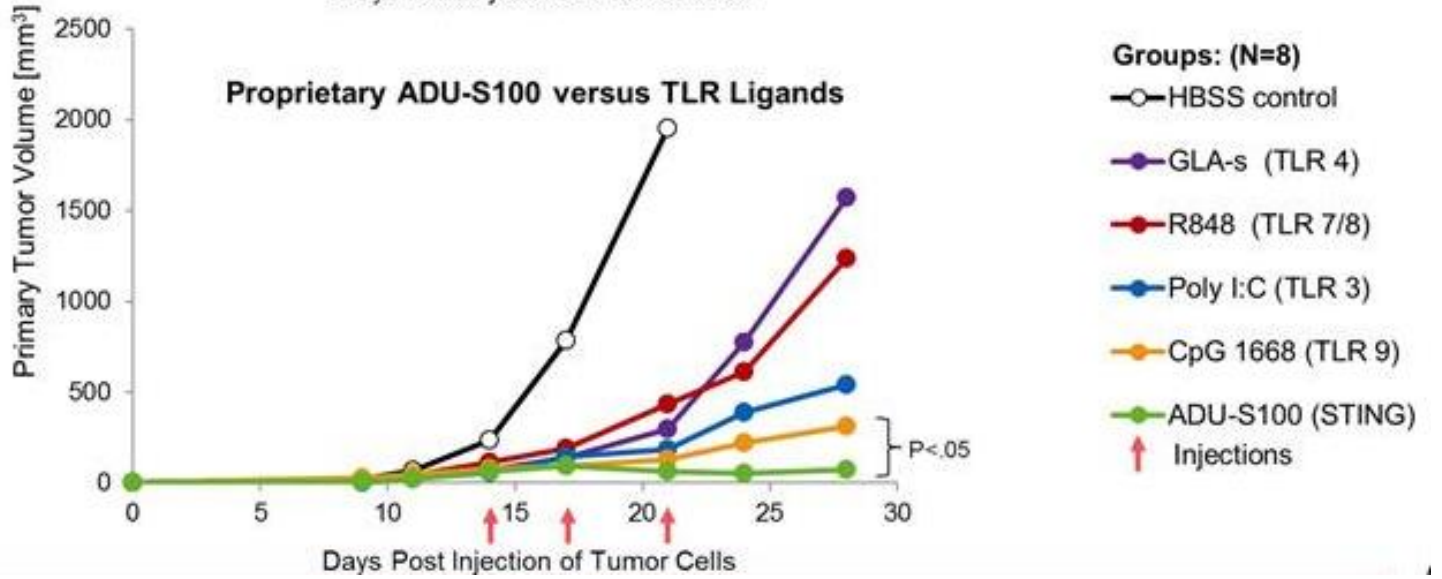
Obeid J, et al. *Semin Oncol.* 2015;42(4):549-561.



Ossenbrug et al. Cell Chem Biol 2017

Intra-lesional TLR and STING agonists induce therapeutic responses in murine B16 melanoma





Courtesy Aduro

Multiple STING and TLR agonists in clinical development

Drug candidate	Companies	Target (drug modality)	Status
MK-1454	Merck & Co.	STING (cyclic dinucleotide)	Phase I monotherapy and combination
ADU-S100	Aduro Biotech/Novartis	STING (cyclic dinucleotide)	Phase I monotherapy and combination
STING agonist	IFM Therapeutics/BMS	STING (cyclic dinucleotide)	Preclinical
STING agonist	Nimbus Therapeutic	STING (small molecule)	Preclinical
NLRP3 agonist	IFM Therapeutics/BMS	NLRP3	Phase I to start in Q1 2018
RGT100	Rigontec/Merck & Co.	RIG-I (oligonucleotide)	Phase I/II
IMO-2125	Idera Pharmaceuticals	TLR9 (oligonucleotide)	Phase I/II

BMS, Bristol-Myers Squibb; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; RIG-I, retinoic acid inducible gene 1; STING, stimulator of interferon genes; TLR9, Toll-like receptor 9.

Clinical trial results of TLR9 agonist monotherapy

Agent	Treatment Arms	Study Phase	Cancer Type	No. Patients	Results	References
PF-3512676	PF-3512676 8 mg *vs.* saline	Phase II randomized	Early stage melanoma	24	In the experimental arm: larger sentinel lymph nodes (SLN), higher SLN leucocytes, higher maturation markers of DC, lower T-reg, increased cytokines	Molenkamp *et al.* [61,62]
PF-3512676	PF-3512676 0.01–5/10 mg	Phase I	BCC and advanced melanoma	10	Local tumor regression, post-treatment cytokines levels reduction, dense intra- and peri-tumoral lymphocytic infiltrates	Hofmann *et al.* [63]
PF-3512676	PF-3512676 6 mg	Phase II	Advanced melanoma	20	PR = 10%, CR = 5%, SD = 15% (DCR = 30%)	Pashenkov *et al.* [64]
PF-3512676	PF-3512676 0.08, 0.12, 0.16, 0.36, 0.54, 0.81 mg/kg	Phase I/II	Metastatic RCC	39	PR = 5%, DCR = 30%	Thompson *et al.* [65]
PF-3512676	PF-3512676 10 mg *vs.* PF-3512676 40 mg *vs.* PF-3512676 40 mg + DTIC 850 mg/m^2 *vs.* DTIC 850 mg/m^2	Phase II randomized	Untreated advanced melanoma	184	Higher ORR (16%) for PF-3512676 40 mg + DTIC 850 mg/m^2 no differences in mTTP and mOS	Weber *et al.* [66]
PF-3512676	PF-3512676 0.2 mg/kg + taxane/platinum *vs.* taxane/platinum	Phase II randomized	Untreated advanced NSCLC	117	Higher ORR for PF-3512676 0.2 mg/kg + taxane/platinum (38% *vs.* 19%) Longer mOS PF-3512676 0.2 mg/kg + taxane/platinum (12.3 *vs.* 6.8 ms)	Manegold *et al.* [67]
PF-3512676	PF-3512676 0.2 mg/kg + CBDCA/TXL *vs.* CBDCA/TXL	Phase III	Untreated advanced NSCLC	828	No significant differences in mOS neither mPFS	Hirsh *et al.* [68]
PF-3512676	PF-3512676 0.2 mg/kg + CDDP/GEM *vs.* CDDP/GEM	Phase III	Untreated advanced NSCLC	839	No significant differences in mOS neither mPFS	Manegold *et al.* [67]

Melisis et al. Biomedicines 2014

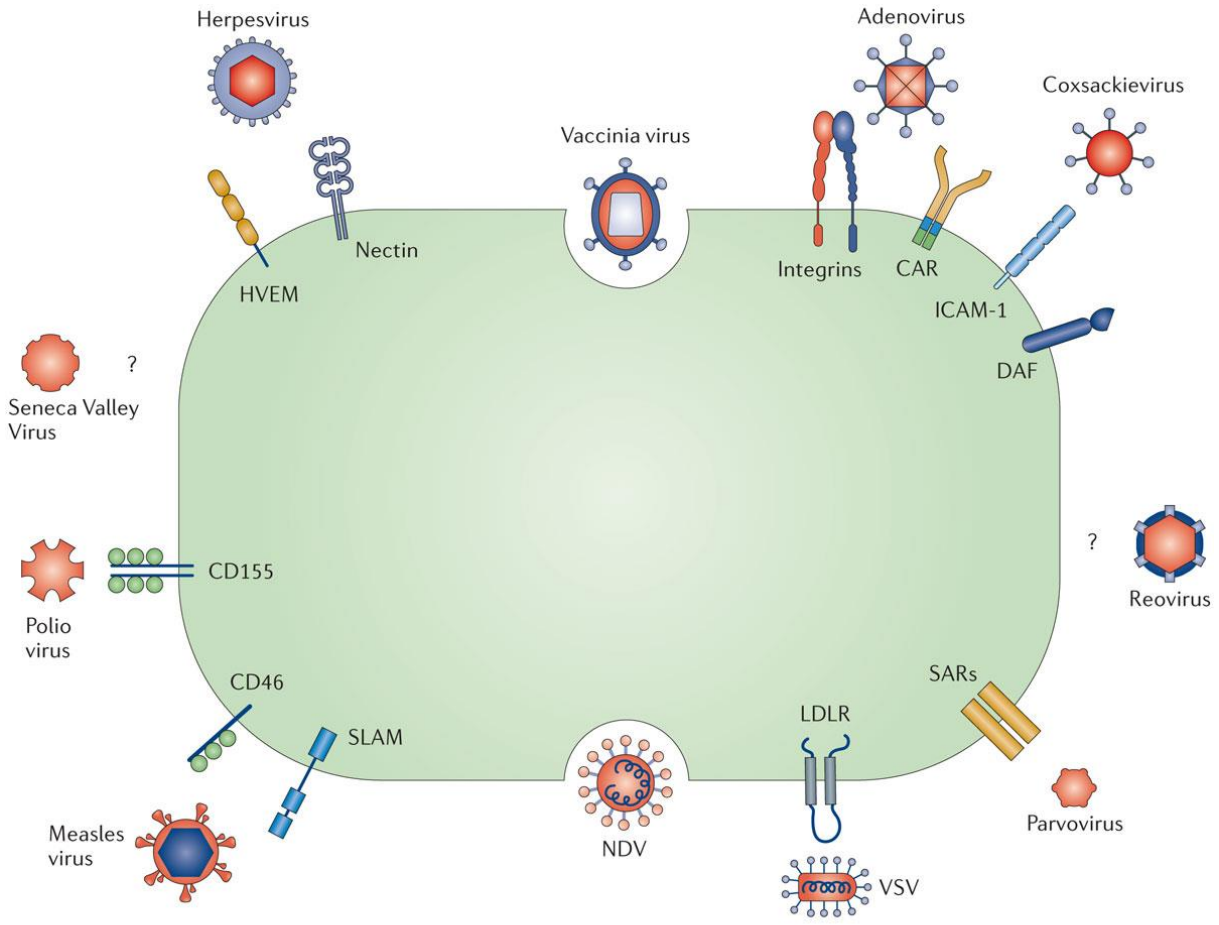
Intratumoral Immunotherapy

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

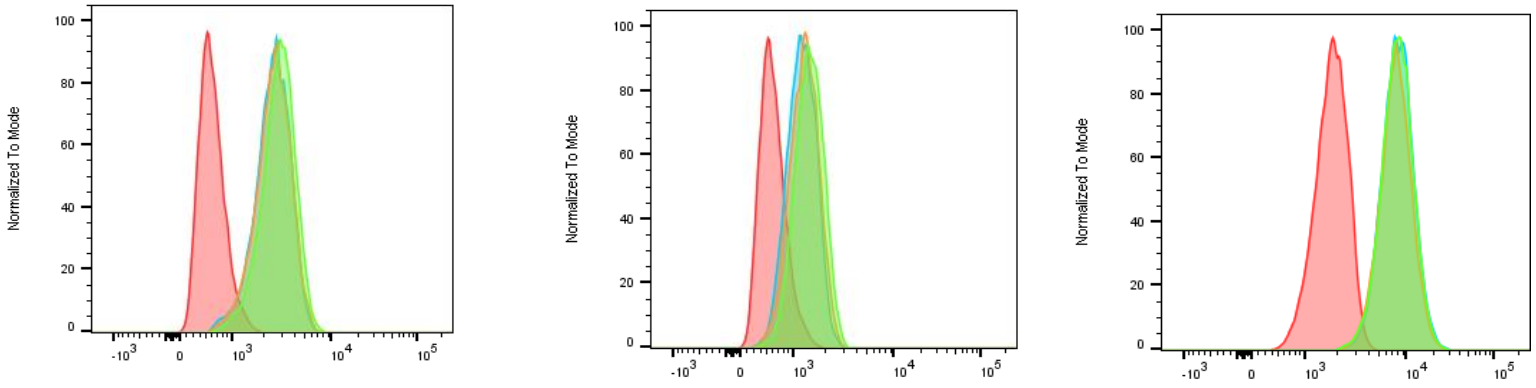


Nature Reviews | Drug Discovery

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

HSV-1 utilizes HVEM, Nectin-1 and Nectin-2 to enter tumor cells

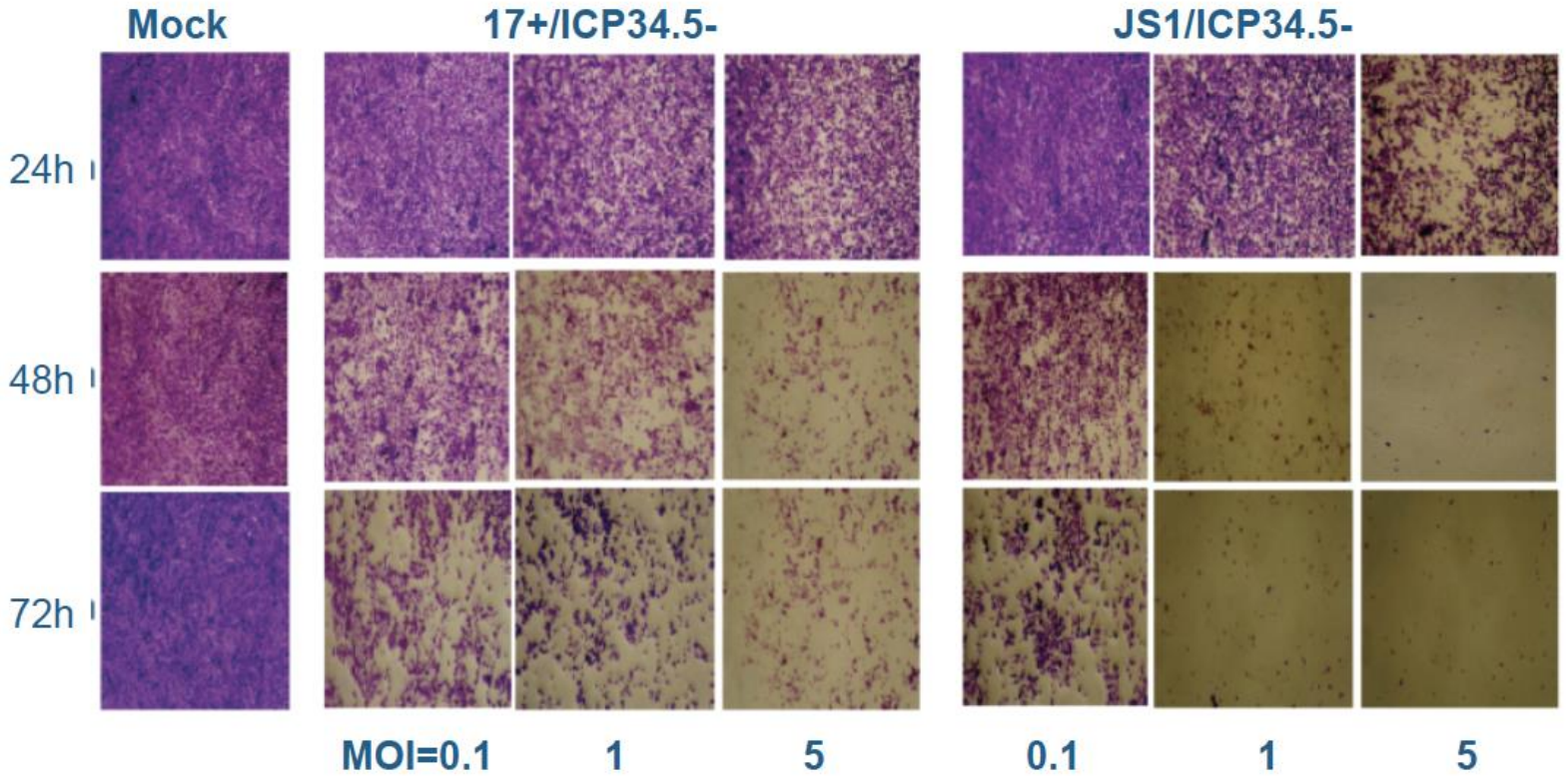
SKMEL5



SKMEL28

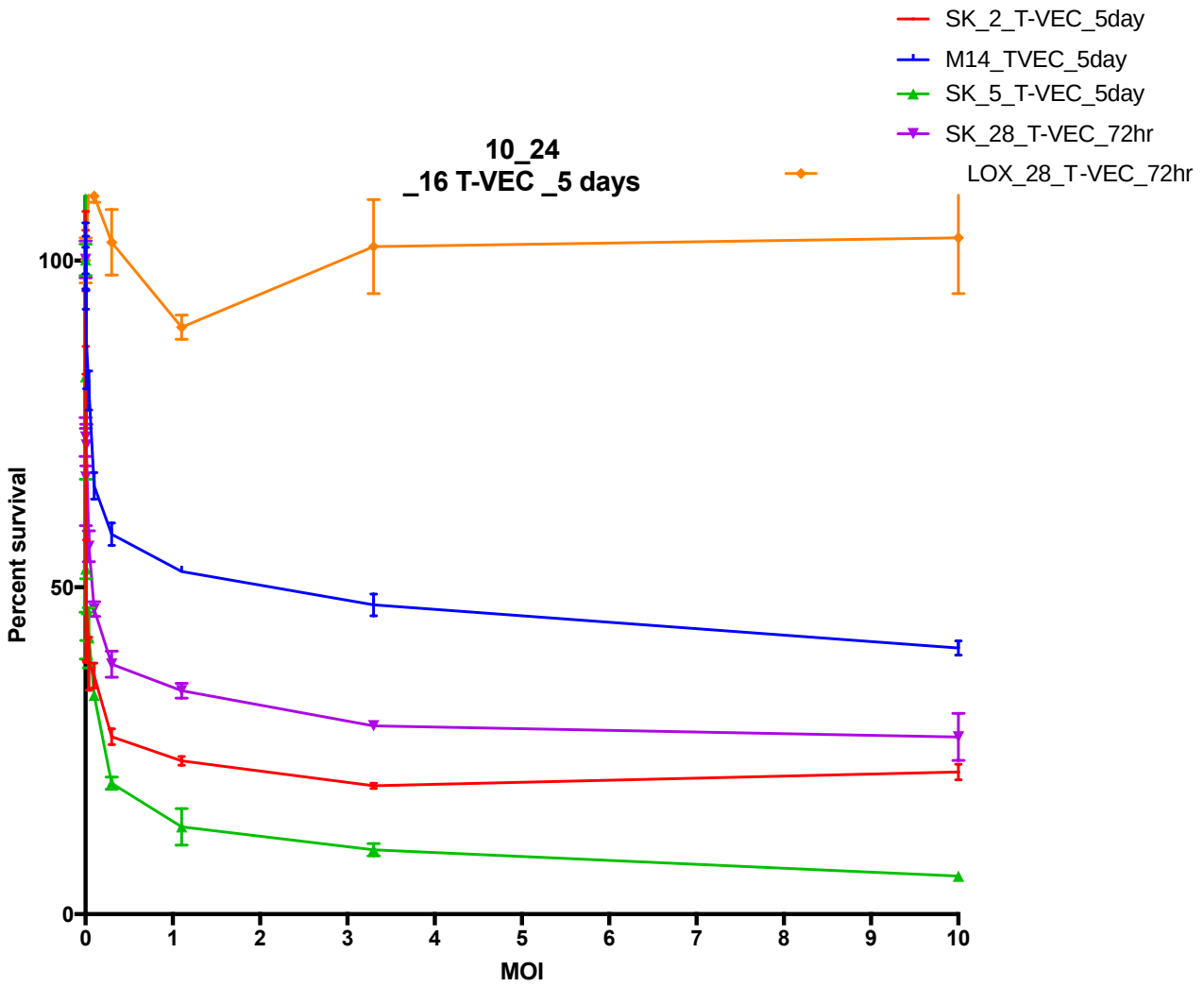
HVEM **Nectin-1** **Nectin-2**

T-VEC induces lysis of SK-MEL-28 melanoma cells in a dose response manner [*In vitro* lysis assay]



Liu et al Gene Therapy 2003

Dose-response lysis of various melanoma cell lines

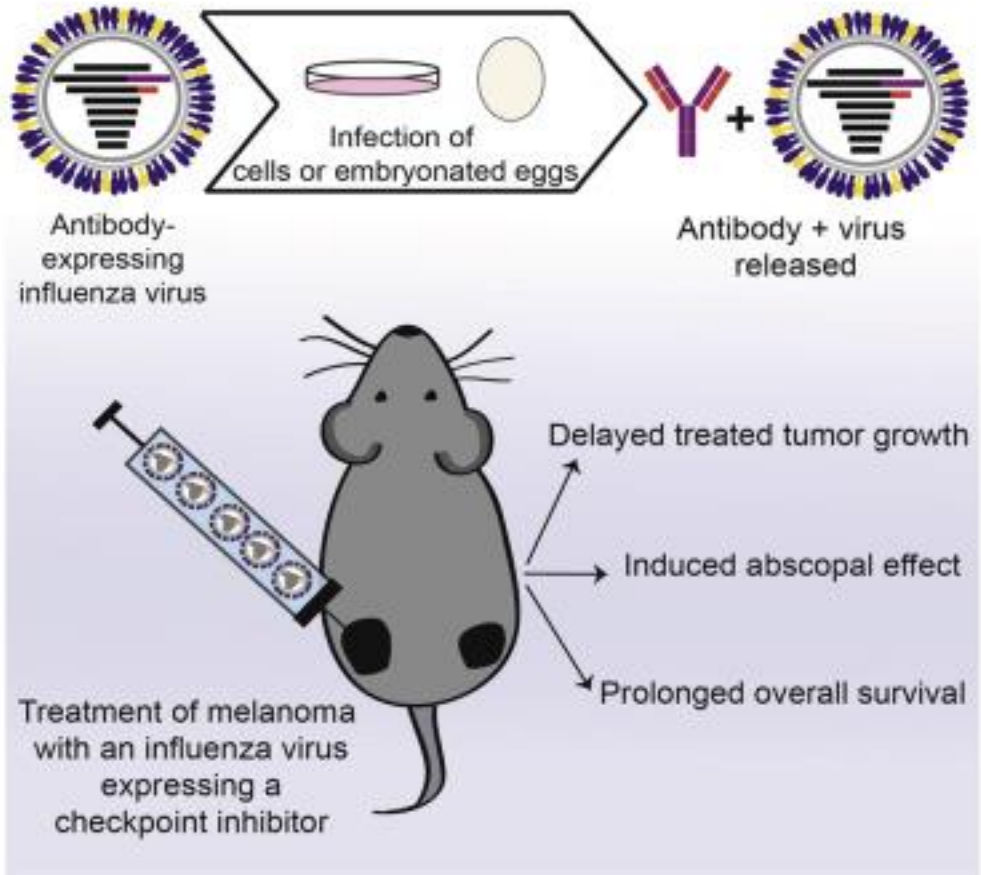


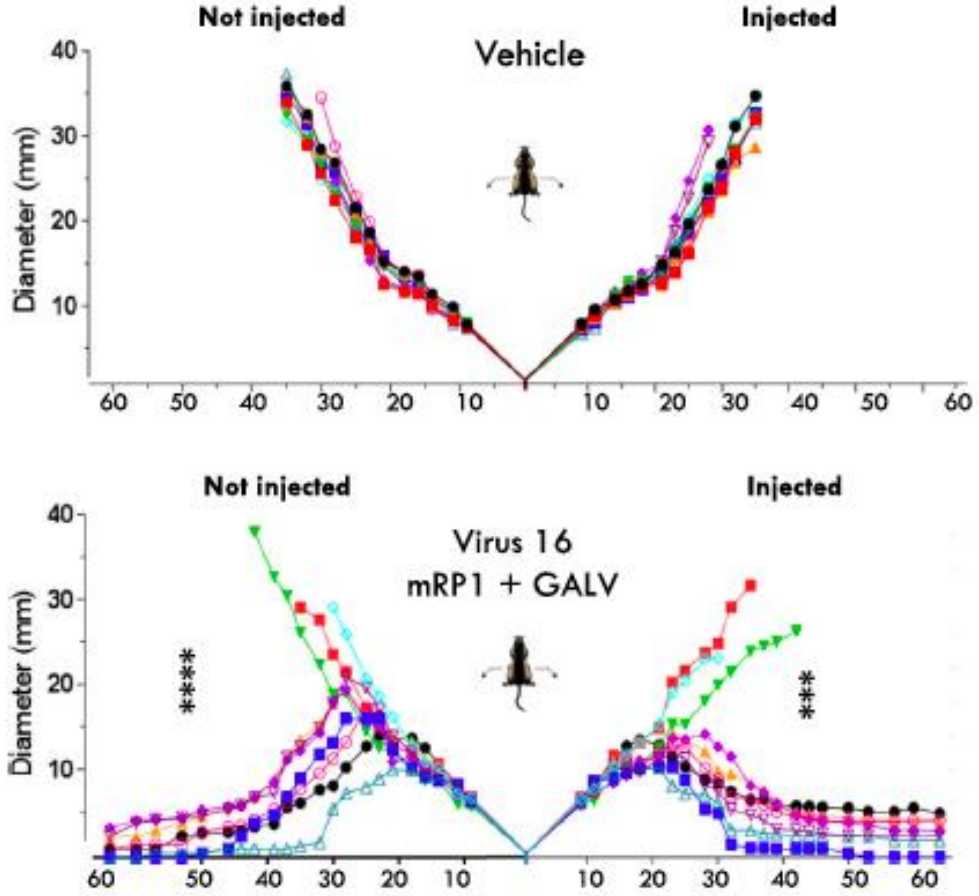
T-VEC induces lysis of human tumor cell lines [*In vitro* lysis across cell lines]

Cell lines	Tissue	Cell survival (%) (MOI=1) 24 hrs	48 hrs	3 days	6 days
A549	Lung cancer	82.5	76.0	55.8	43.1
H460	Lung cancer	65.2	64.0	44.0	27.6
CALU-1	Lung cancer	71.1	60.0	41.9	40.4
PANC-1	Pancreatic cancer	74.6	57.6	24.1	9.4
MIA PACA-2	Pancreatic cancer	66.5	38.5	18.6	1.4
CAPAN-1	Pancreatic cancer	81.0	42.2	56.6	20.3
BxPC-1	Pancreatic cancer	57.6	15.1	16.1	8
HCT116	Colorectal cancer	65.7	27.4	14	1.1
HT29	Colorectal cancer	51.6	22.0	24.3	3.9
SW620	Colorectal cancer	80.4	66.8	45.0	3.9
COLO205	Colorectal cancer	49.8	20.0	9.7	3.1

Liu et al Gene Therapy 2003

Intratumoral therapy should report injected and un-injected tumor responses

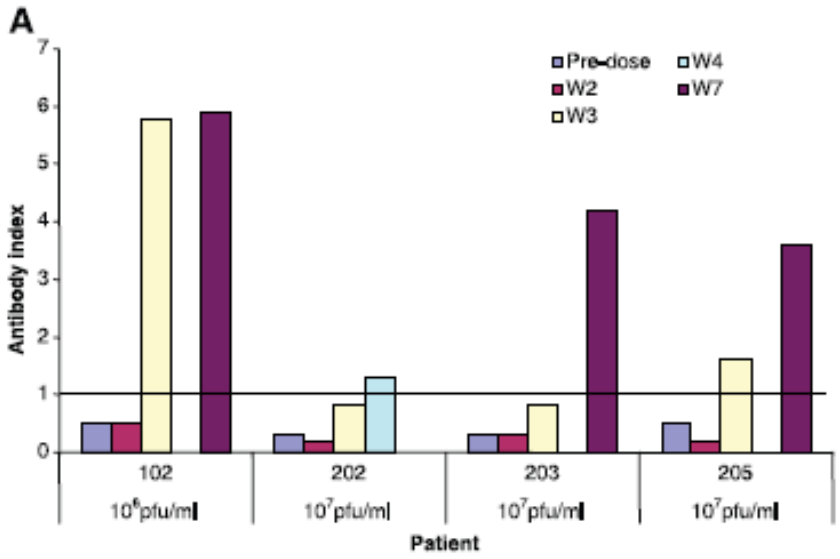




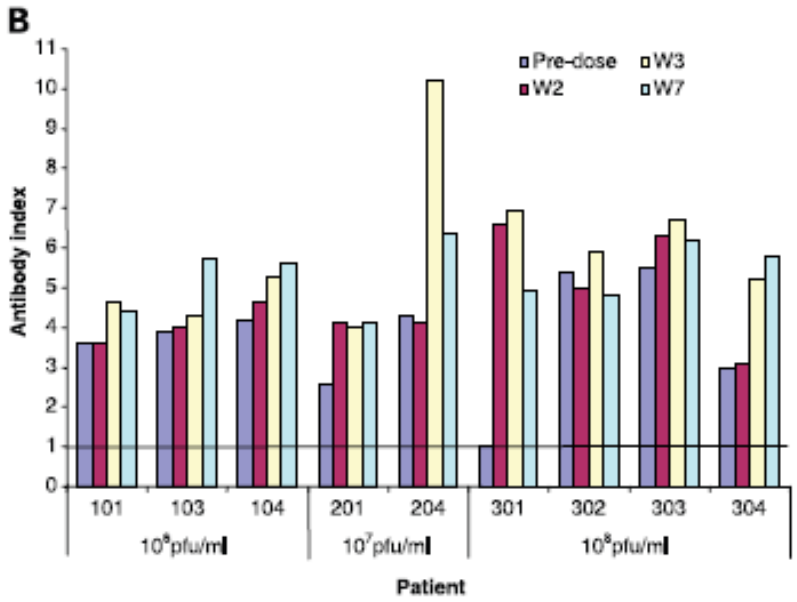
Hamilton et al. Cell 2018
Thomas et al. JITC 2019

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

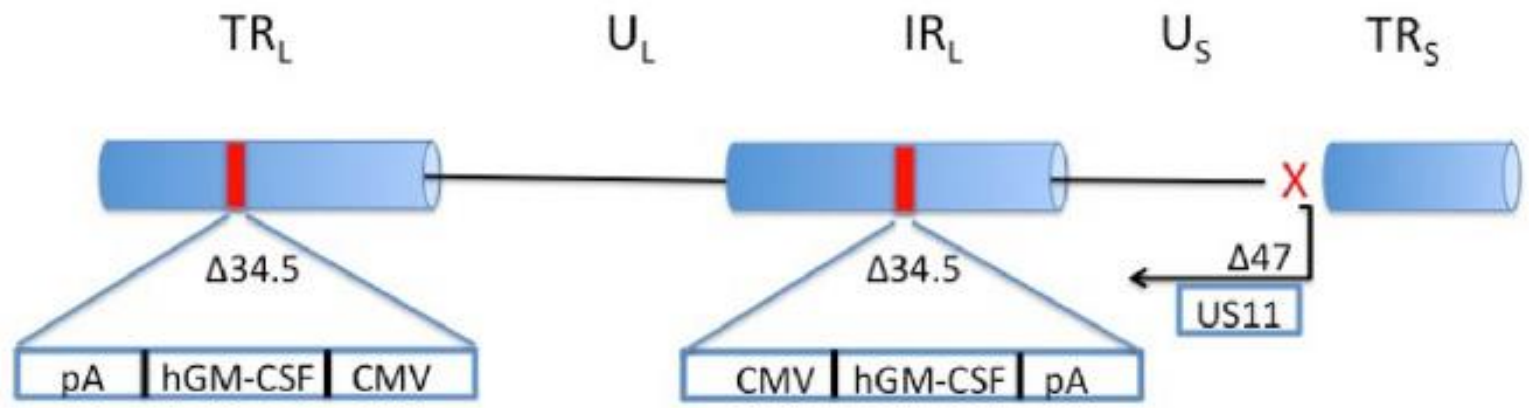
Clinical Issues

- 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)

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

Lessons Learned from Talimogene laherparepvec (T-VEC)

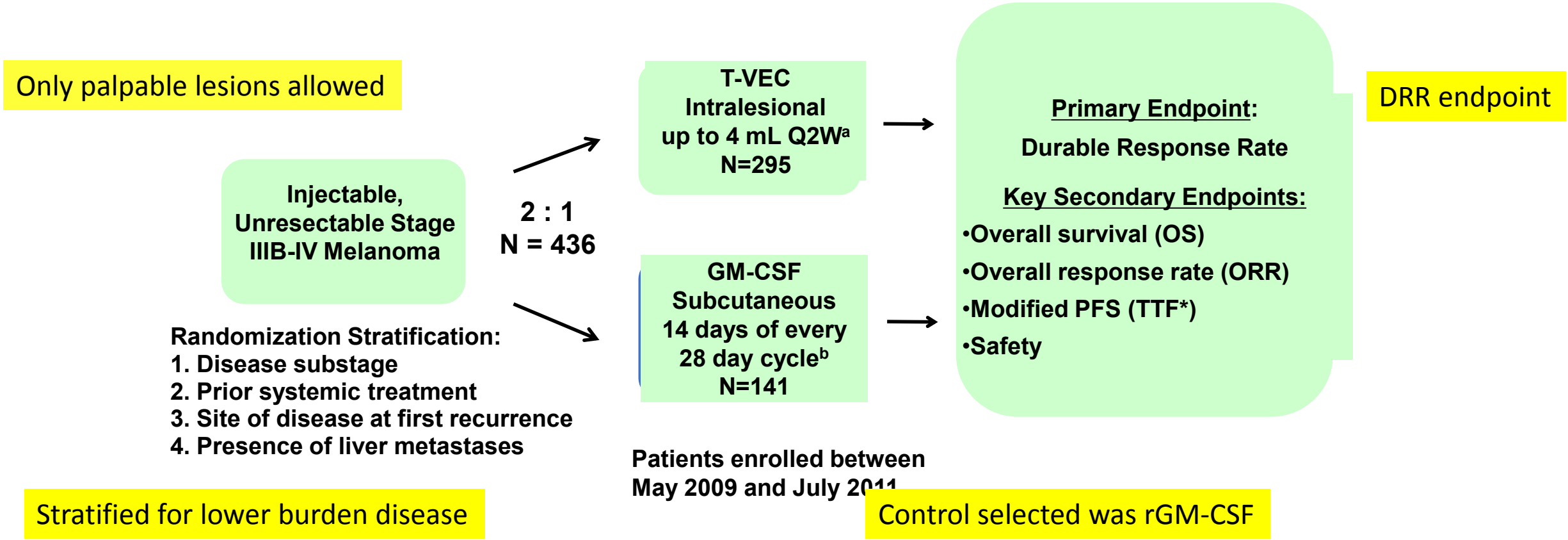


- Modified HSV-1
- 26% ORR and 16% DRR in a Phase III trial
- T-VEC is approved in the U.S., Europe and Australia

Modifications	Rationale
JS-1 strain	Improved Cancer Cell Lysis
Deletion of ICP34.5	Cancer Cell Specific Replication
Early Expression of US11	Cancer Cell Specific Replication
Deletion of ICP47	Permits Antigen Presentation
Insertion of GM-CSF	Augments anti-tumor Immune response

Bommareddy et al. Am J Clin Dermatol 2016

OPTiM Phase III Study Design



Patients were to remain on treatment for at least 24 weeks despite progression (unless intolerability or investigator decision to start new therapy)

[a] Dosing of T-VEC was ≤ 4 mL x10^6 pfu/mL once, then after 3 weeks, ≤ 4 mL x10^8 pfu/mL Q2W

[b] Dosing of GM-CSF was 125 µg/m^2 subcutaneous daily x14 days of every 28 day cycle.

Treated 24 weeks with PD

*Andtbacka et al. ASCO 2013; LBA9008

Volume determination for T-VEC

Lesion size (longest diameter)	T-VEC injection volume
>5 cm	Up to 4 mL
>2.5–5 cm	Up to 2 mL
>1.5–2.5 cm	Up to 1 mL
>0.5–1.5 cm	Up to 0.5 mL
≤0.5 cm	Up to 0.1 mL

Abbreviation: T-VEC, talimogene laherparepvec.

Starting dose 10^6 PFU/mL
Maintenance dose 10^8 PFU/mL every 2 weeks

Volume associated with tumor diameter

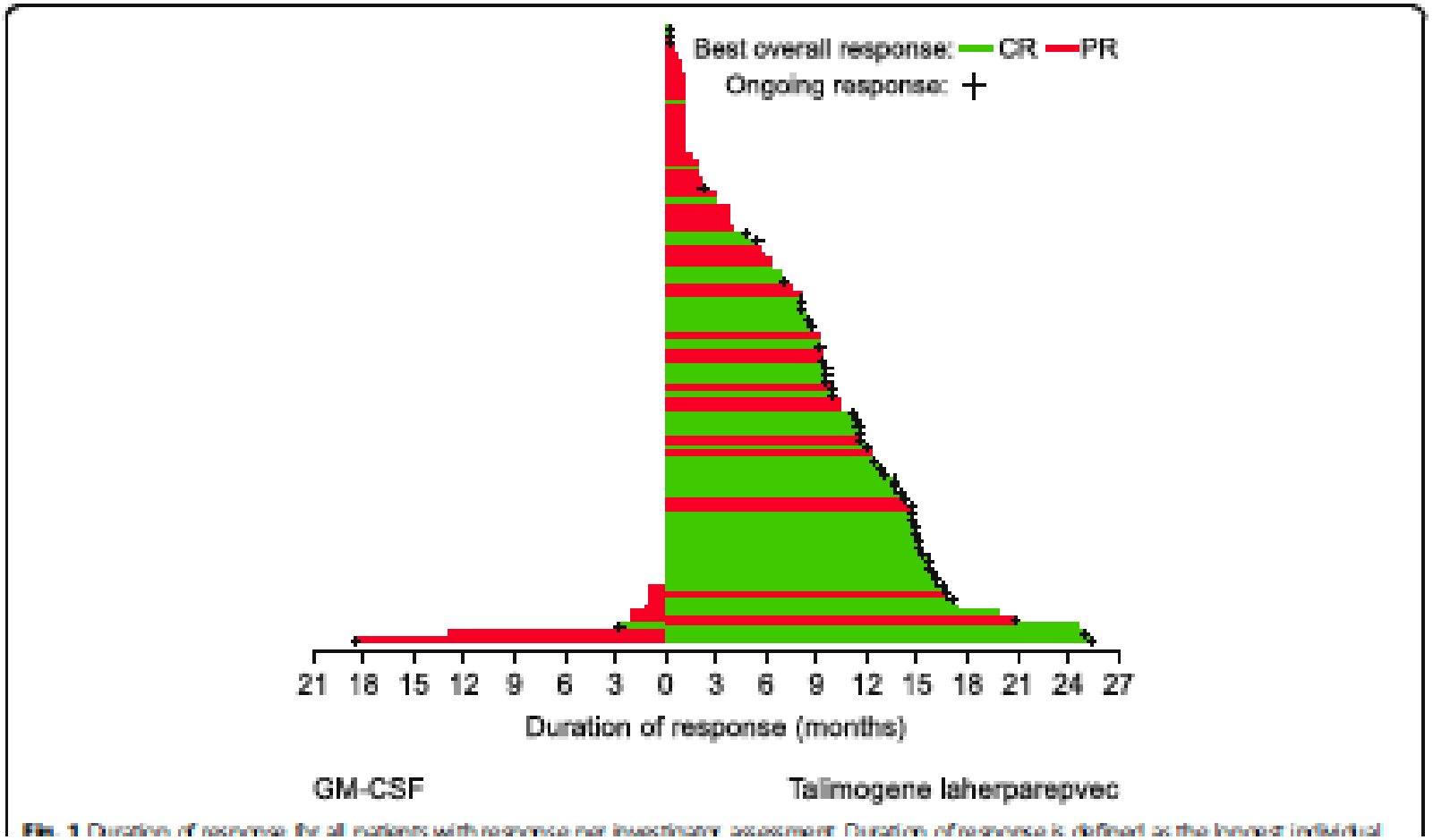
T-VEC improves objective and durable response rates

ITT Set	GM-CSF (N=141)	T-VEC (N= 295)	**Treatment Difference (T-VEC – GM-CSF)**
Overall Response Rate (95% CI)	5.7% (1.9, 9.5)	26.4% (21.4, 31.5)	20.8% (14.4, 27.1) *P* < 0.0001[a] descriptive
CR	0.7%	10.8%	
PR	5.0%	15.6%	

ITT Set	GM-CSF (N=141)	T-VEC (N= 295)	**Treatment Difference (T-VEC – GM-CSF)**
Durable Response Rate	2.1%	16.3%	14.1% 95% CI: (8.2, 19.2) *P* < 0.0001[a]

Kaufman et al. JCO 2015

Final analysis of OPTiM trial shows sustained clinical benefit



Updated ORR:

T-VEC 31.5%

GM-CSF 6.4%

P<0.0001

Updated DRR:

T-VEC 19.3%

GM-CSF 1.4%

P<0.0001

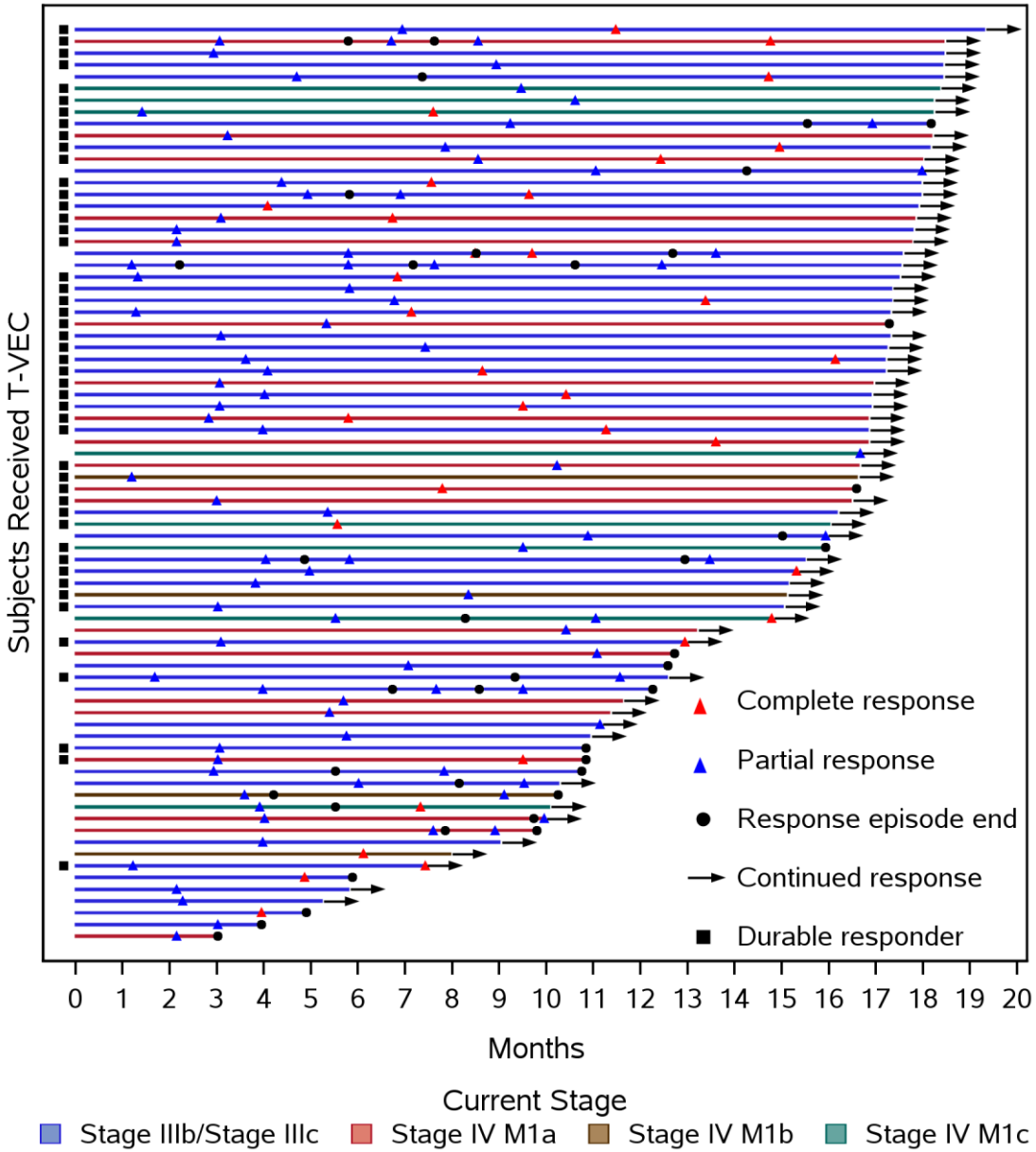
Updated DCR

T-VEC 76.3%

GM-CSF 56.7%

Andtbacka et al. JITC 2019

Time to Response And Duration of Response



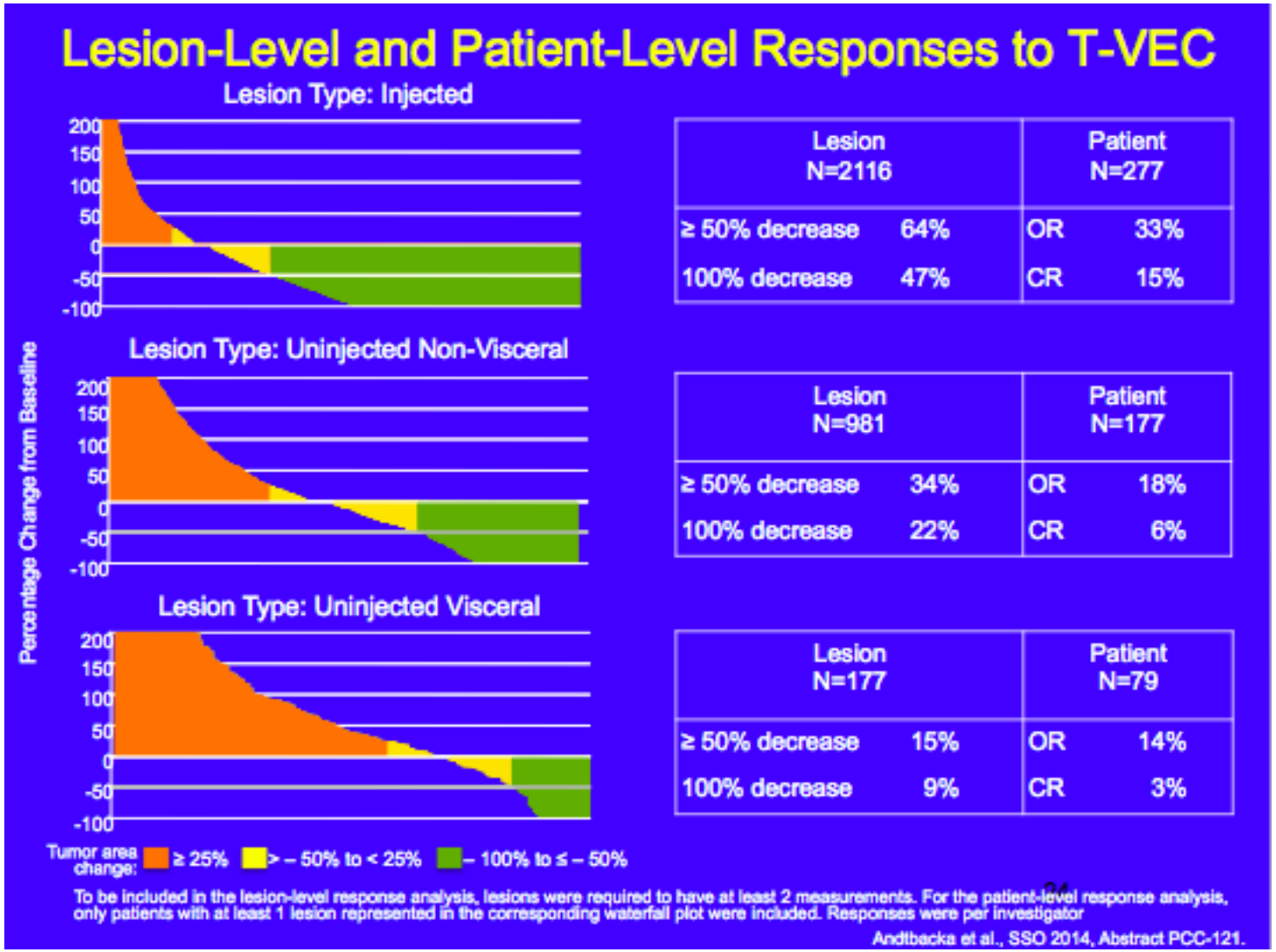
- To be a durable responder, patient had to have response of at least 6 continuous months

- Patients were to continue treatment beyond progression, allowing for re-initiation of response after progression

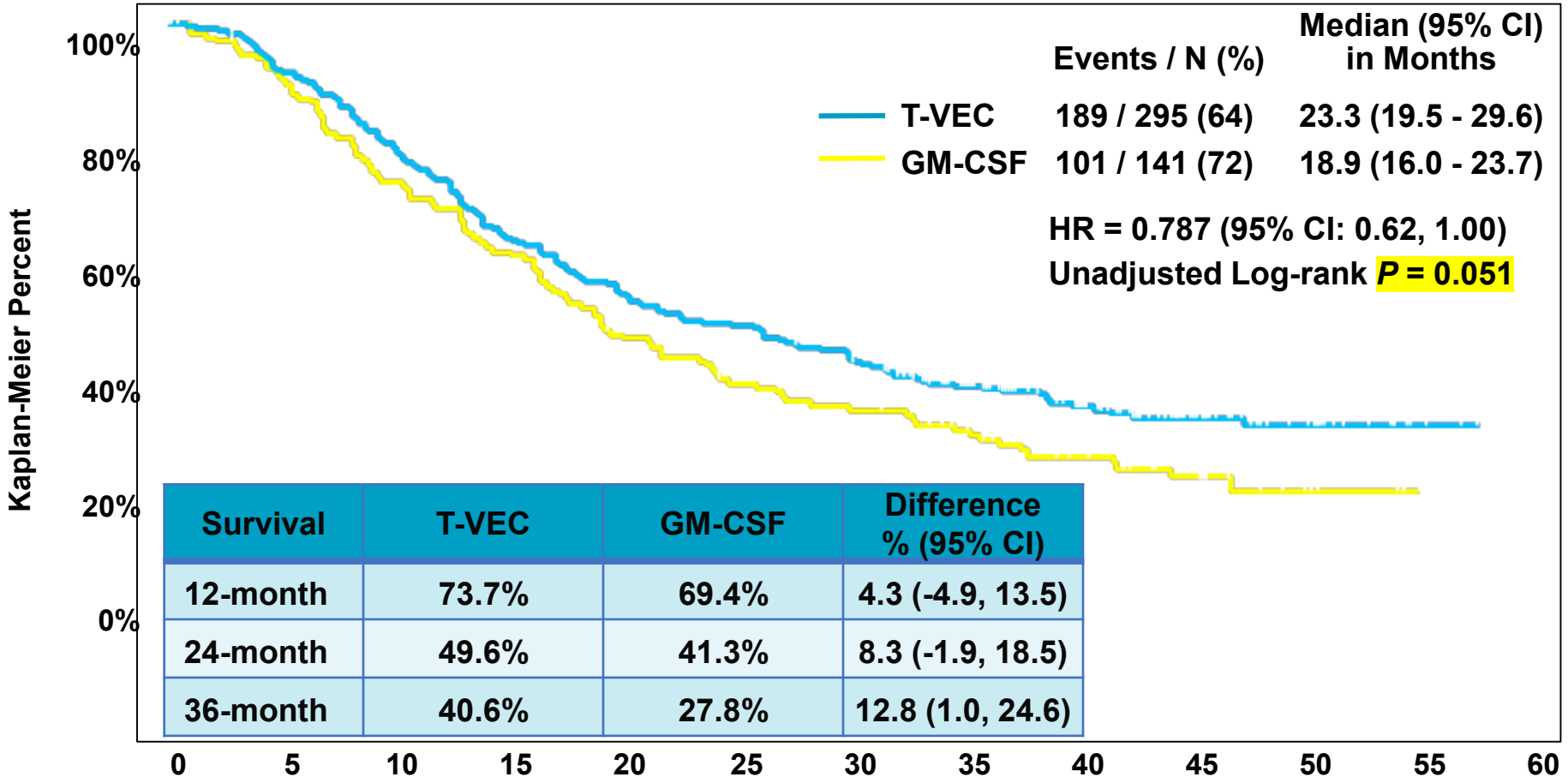
- PD displayed when it represents the end of an objective response. PD also occurred prior to objective responses in many cases (not shown).

•54% ORR and 48% DRR exhibited interval progression before achieving response

Tumor regression in injected lesions is greater than in non-injected lesions



T-VEC improved overall survival



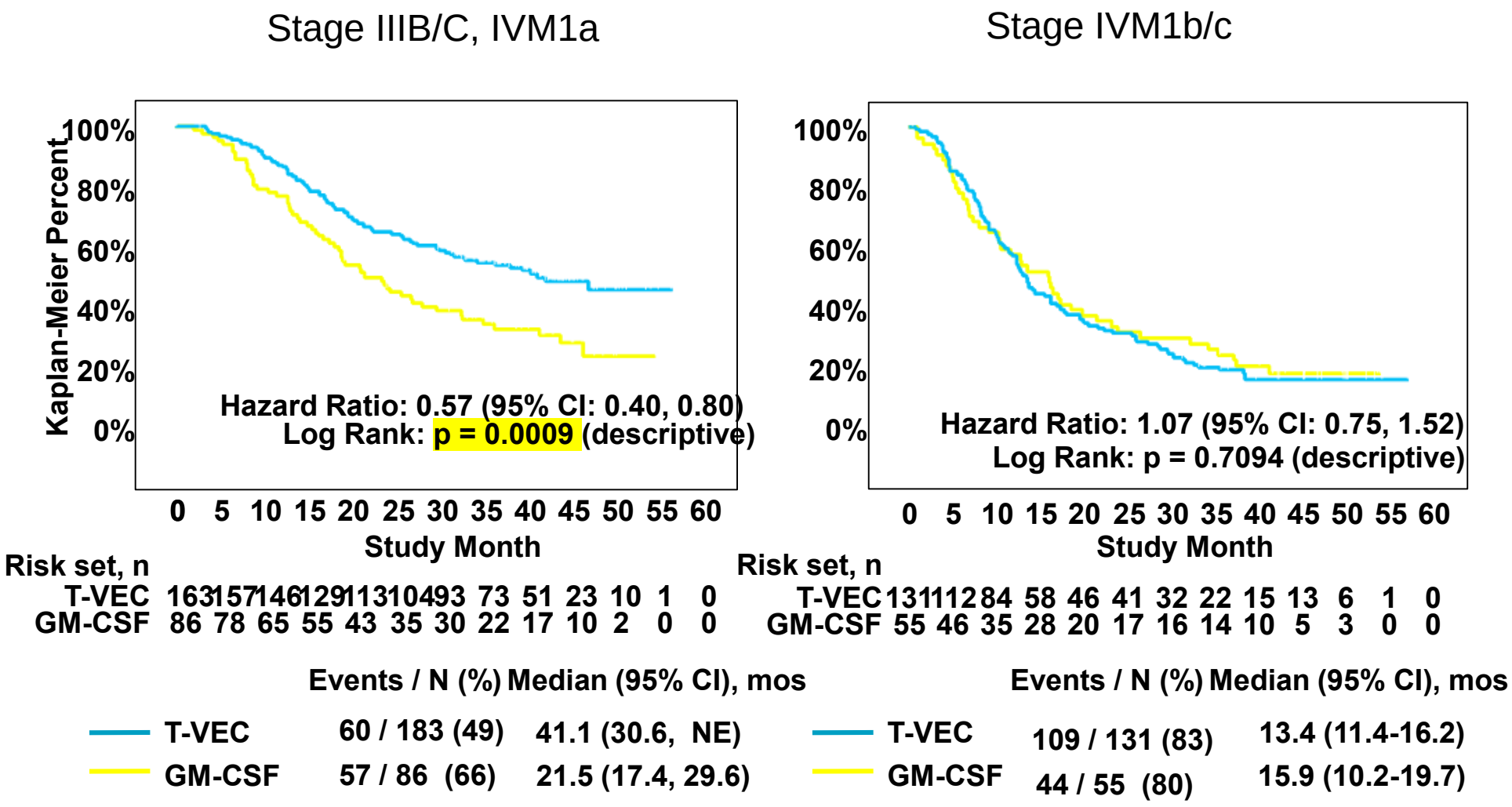
Survival	T-VEC	GM-CSF	Difference % (95% CI)
12-month	73.7%	69.4%	4.3 (-4.9, 13.5)
24-month	49.6%	41.3%	8.3 (-1.9, 18.5)
36-month	40.6%	27.8%	12.8 (1.0, 24.6)

Patients at risk:

T-VEC	141	124	100	83	63	52	46	36	27	15	5	0	0
GM-CSF	295	269	230	187	159	145	125	95	66	36	16	2	0

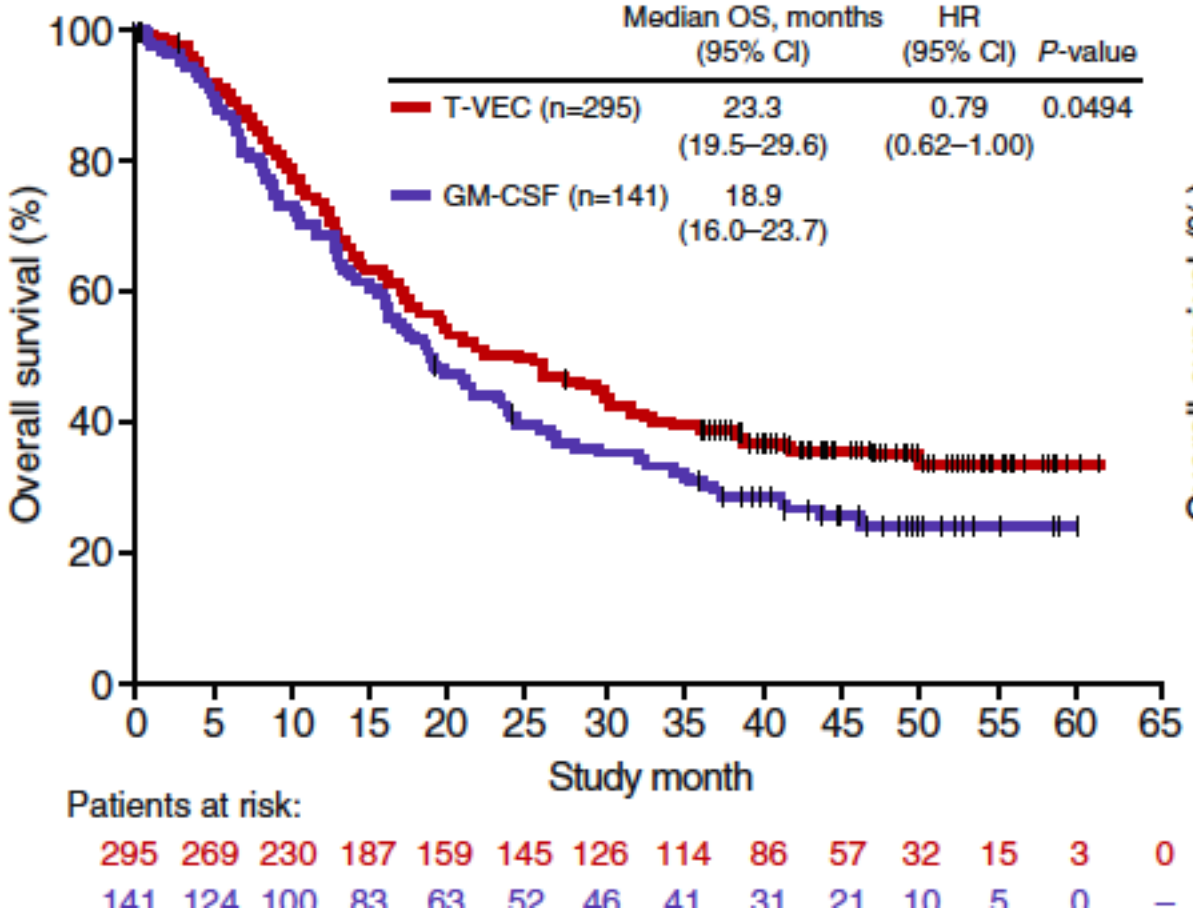
Andtbacka, Kaufman, et al. JCO 2015

OS subgroup analysis by disease stage

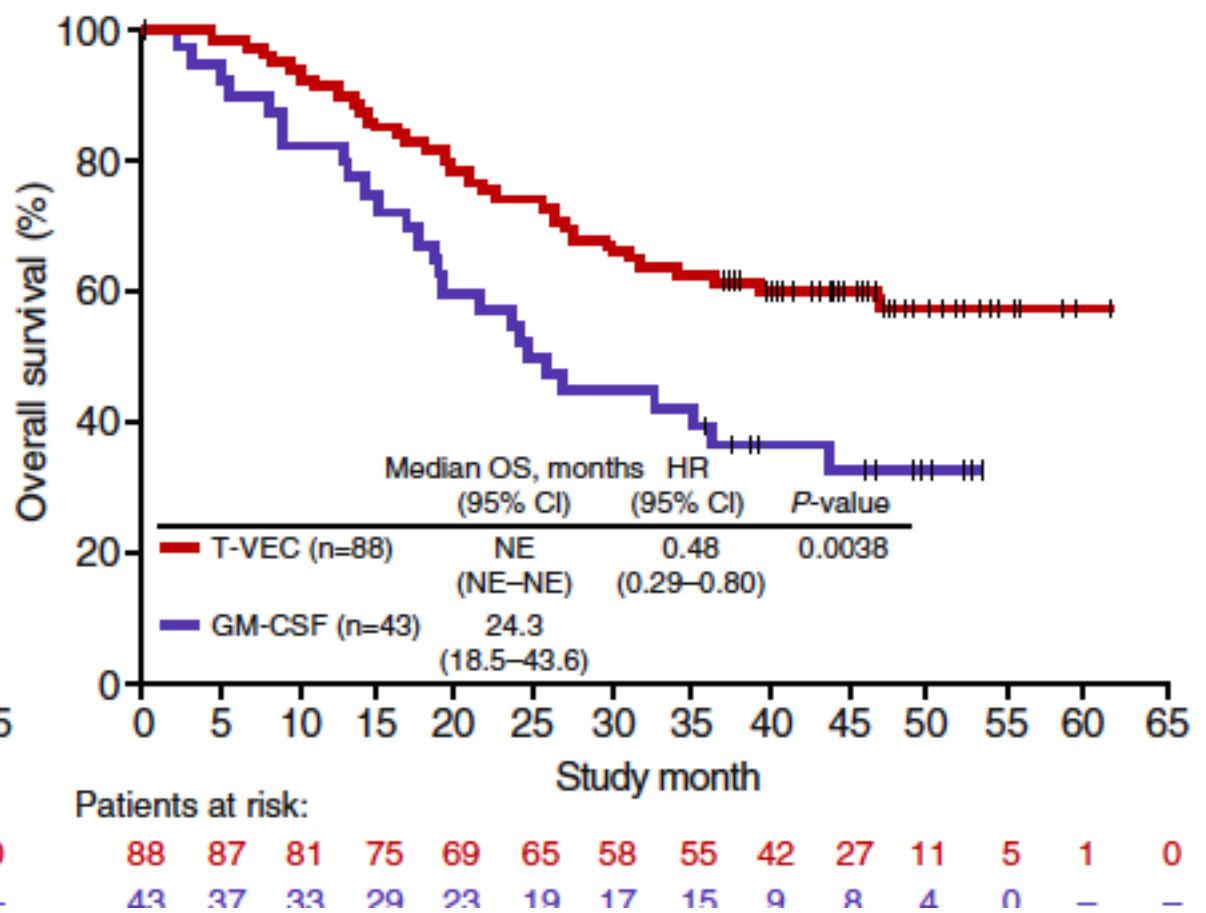


Andtbacka Kaufman, et al. JCO 2015

OPTiM shows sustained OS benefit at 49 months median follow-up



ITT Population



Stage III-IVM1a

Andtbacka et al. JITC 2019

Intratumoral Immunotherapy

Integrating Into Combination Therapy

Initial results of SD-101 and pembrolizumab

Best Overall Response Rate (ITT)	2 mg/lesion (N=45)	8 mg/lesion (N = 41)
Objective response rate (ORR), n (%) (95% CI)	**34 (76)** (61, 87)	**20 (49)** (33, 65)
Complete response	8 (18)	4 (10)
Partial response	26 (58)	16 (39)
Stable disease	2 (4)	7 (17)
Progressive disease	5 (11)	9 (22)
Not evaluable†	4 (9)	5 (12)
Time to response, median (months)	2.2	2.3
Duration of response (DOR), median (months) (95%CI)	not reached (NE, NE)	not reached (14.2, NE)

† Patients discontinued prior to first scan: 2 mg—clinical progression (n=3), consent withdrawn (n=1); 8 mg—clinical progression (n=2), irAE/AE (n=2), withdrew consent (n=1). NE, not estimable; ITT, intent to treat

Note: The concordance between blinded central assessment and investigator assessment on a subset of the 2 mg group (n=38) was 89%

TLR9 agonist

- ORR in patients with BRAF mutant tumors who received 2 mg/lesion (n=18) was 61%
- ORR in patients with PD-L1 negative tumors who received 2 mg/lesion (n=14) was 79%

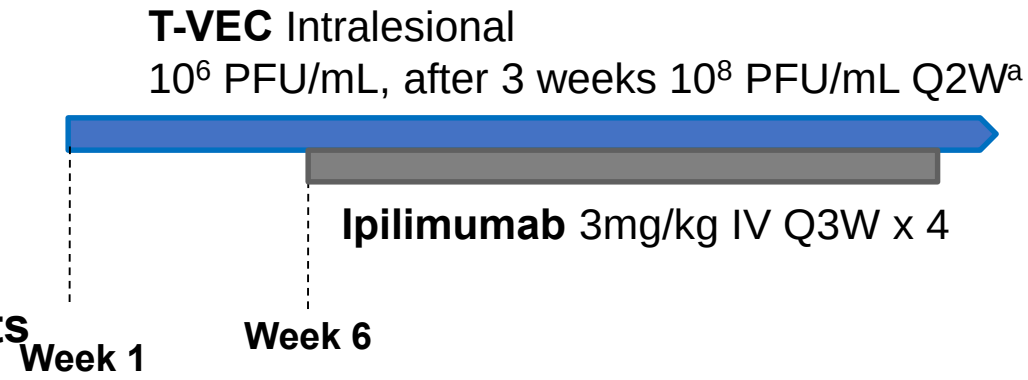
Long et al. ESMO 2018
Milhem et al. ASCO abstract CT144 2019

Study Schema for the phase 1b/II trial of T-VEC and Ipilimumab

Phase I: N = 19
Phase 2: N = 198

Unresectable Stage IIIB-IV Melanoma

- **Injectable**
- **Treatment naive**
- **ECOG PS 0 or 1**
- **No evidence of CNS mets**



T-VEC dosing until CR, all injectable tumors disappeared, PD per irRC, or intolerance whichever comes first.

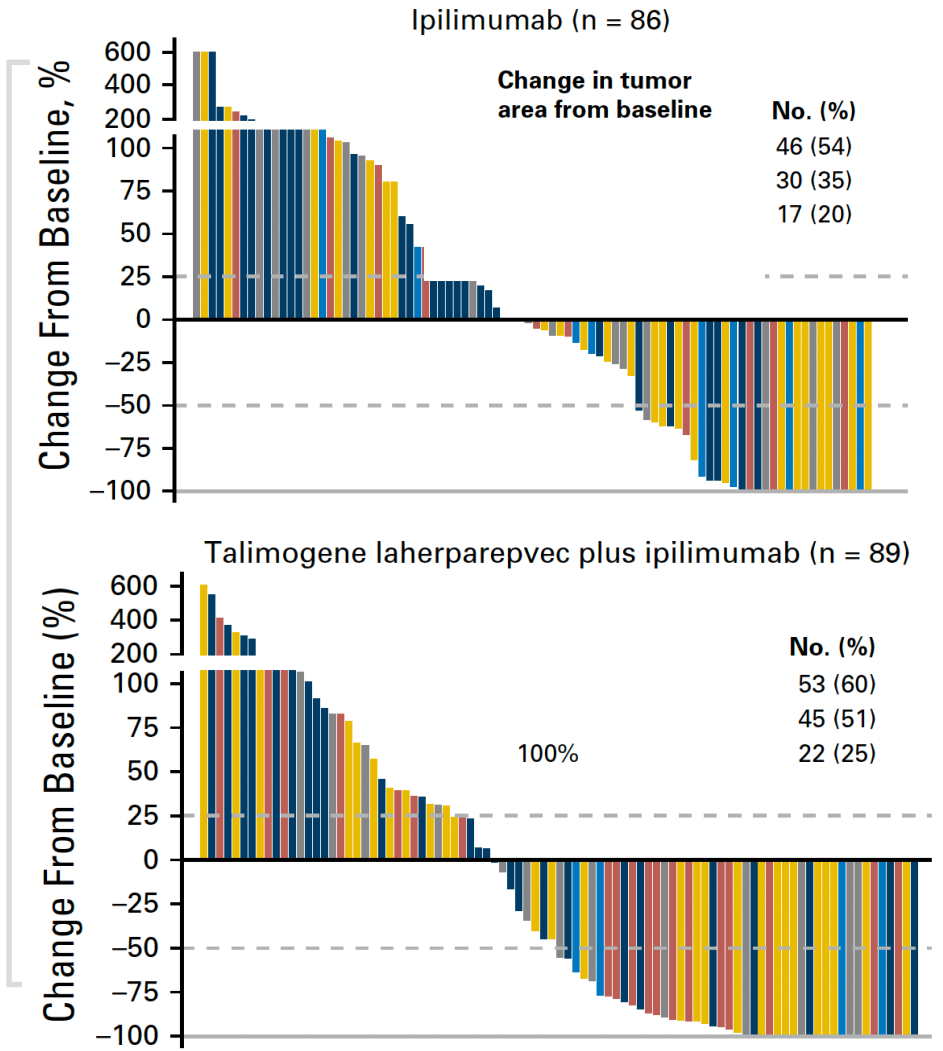
Primary Endpoint (Ph 1B):	Incidence of dose-limiting toxicities (DLTs)
Primary Endpoint (Ph II):	ORR determined by irRC
Key Secondary Endpoints:	BOR, PFS, DoR, time to response, safety

[a] Dosing of T-VEC was δ 4 mL × 10^6 PFU/mL once, then after 3 weeks, δ 4 mL × 10^8 PFU/mL Q2W.

Collichio et al. SMR 2014, Zurich

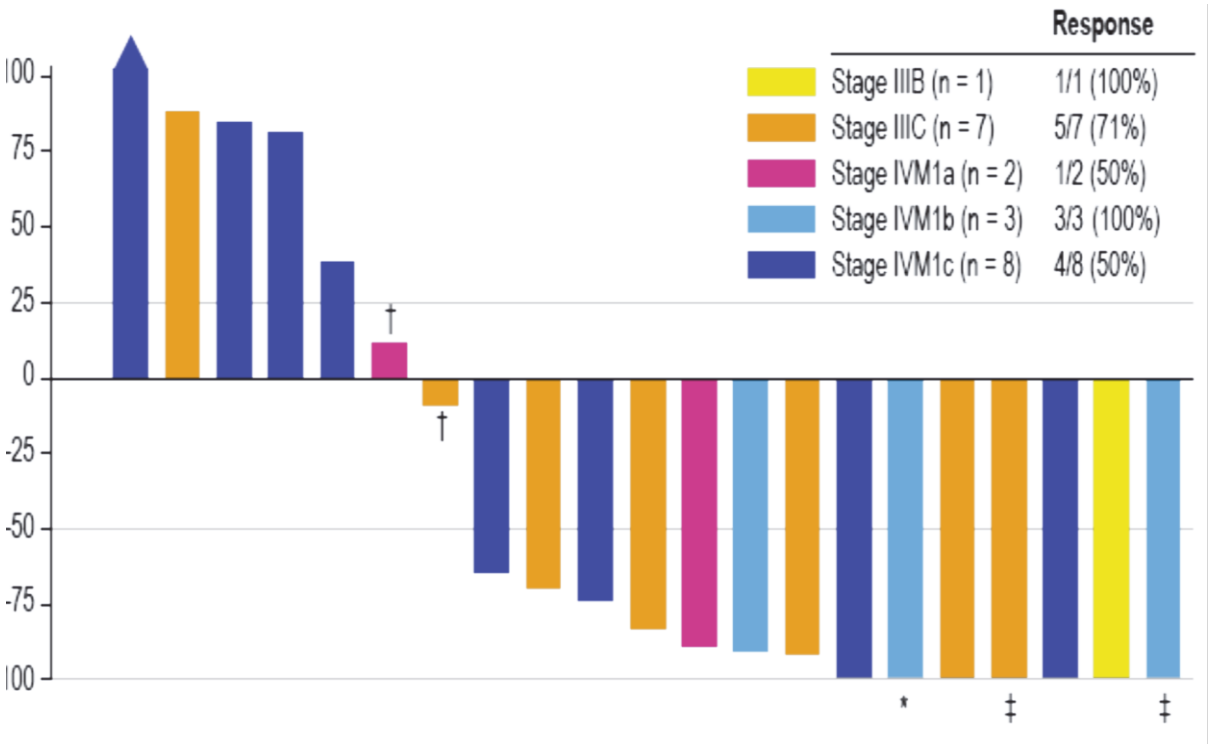
Randomized Phase 2 Clinical Trial: T-VEC + ipilimumab improves ORR

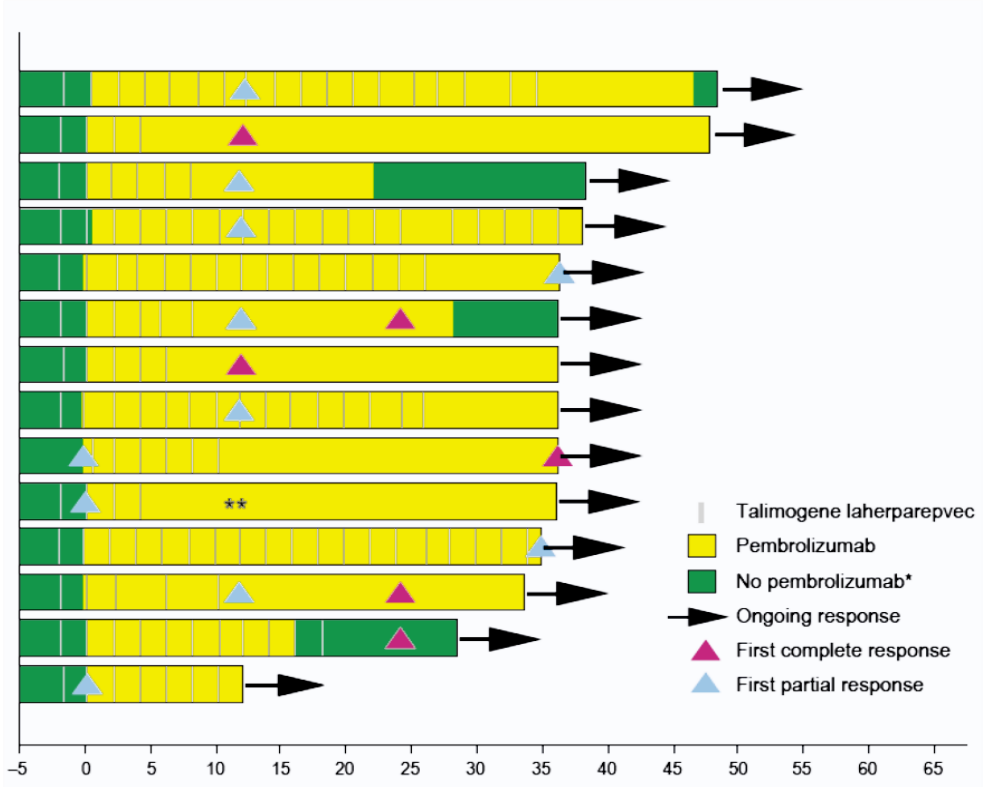
- T-VEC + ipilimumab vs. ipilimumab alone Stage IIIb-IVM1c melanoma
- Response rates (N=198) more than doubled with T-VEC + ipilimumab vs. ipilimumab alone (38% vs. 18%)
- For visceral lesions (none injected), the response rate was 35% for T-VEC +ipilimumab vs. 14% for ipilimumab alone
- No additional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



Chesney et al JCO, 2017

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

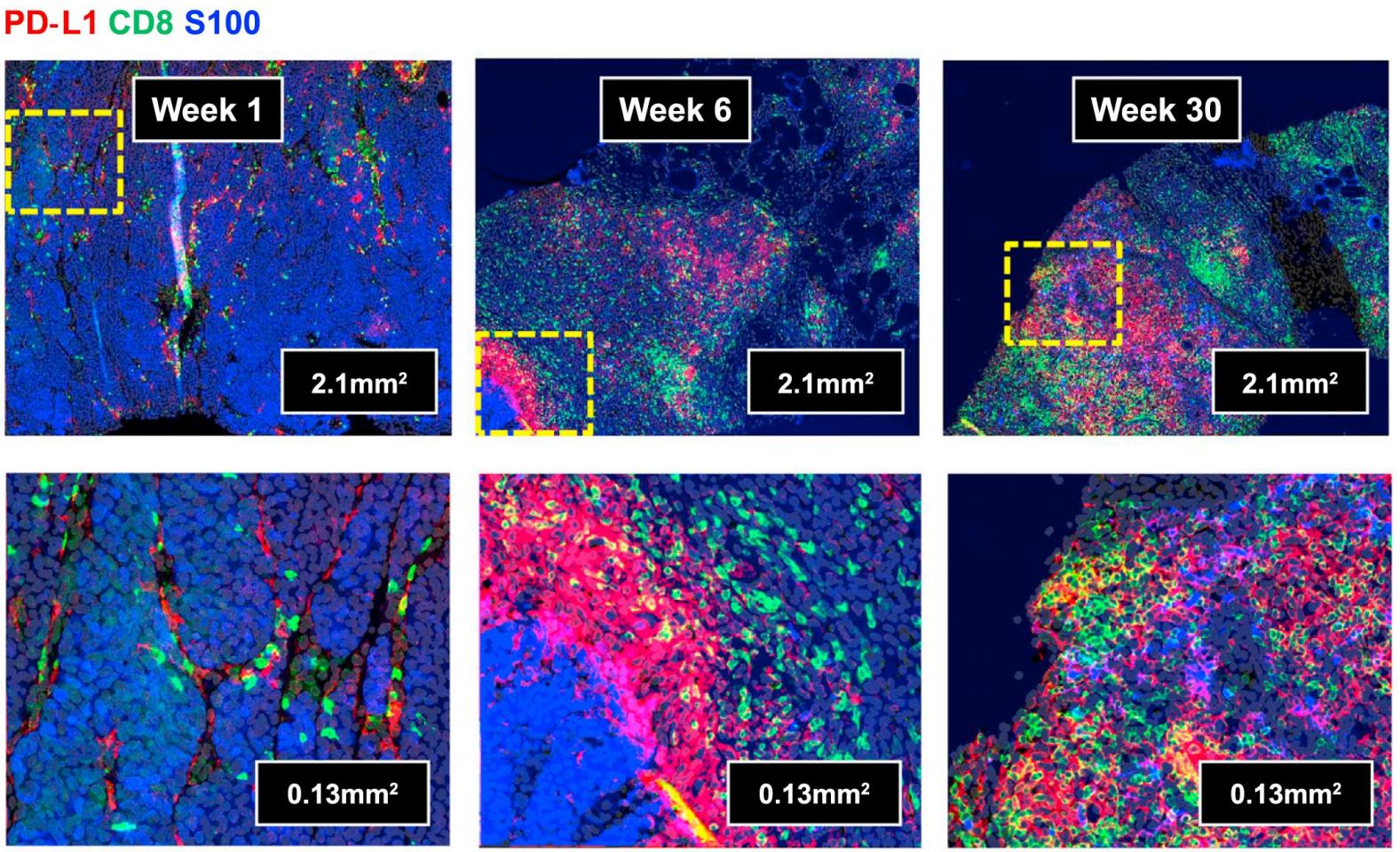




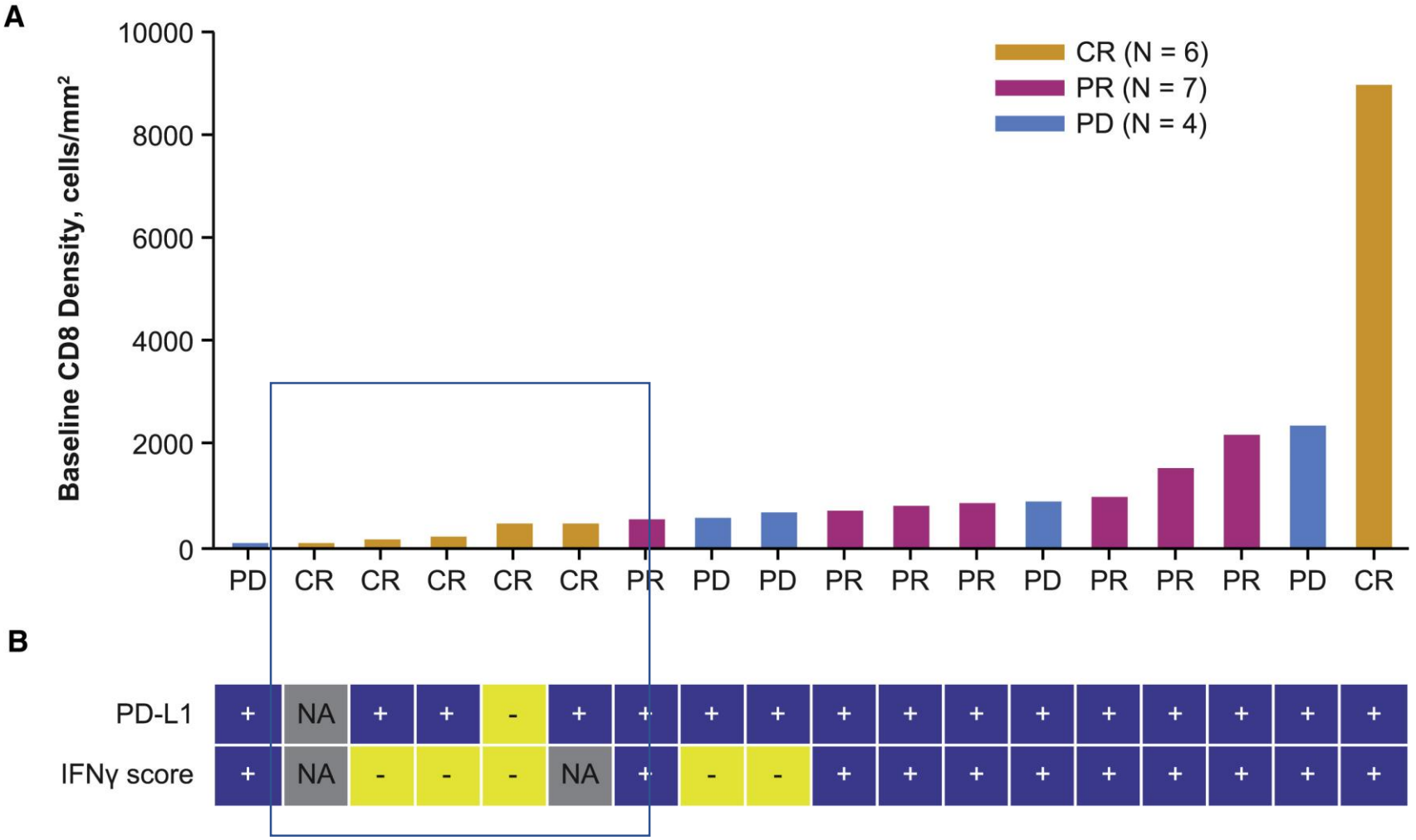
Without added toxicity

Ribas et al. Cell 2017

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

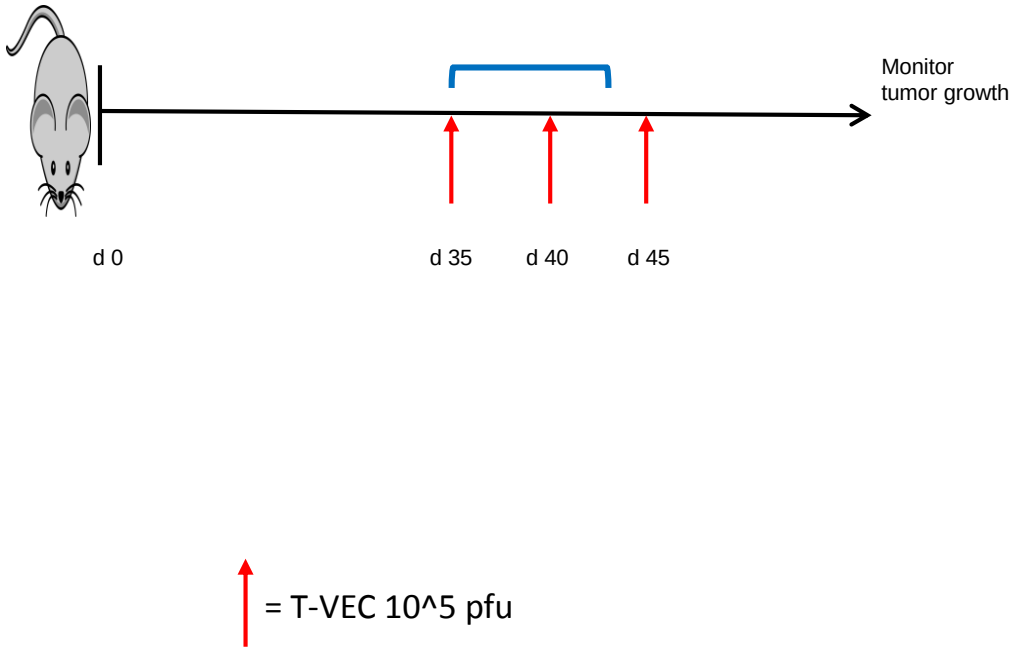


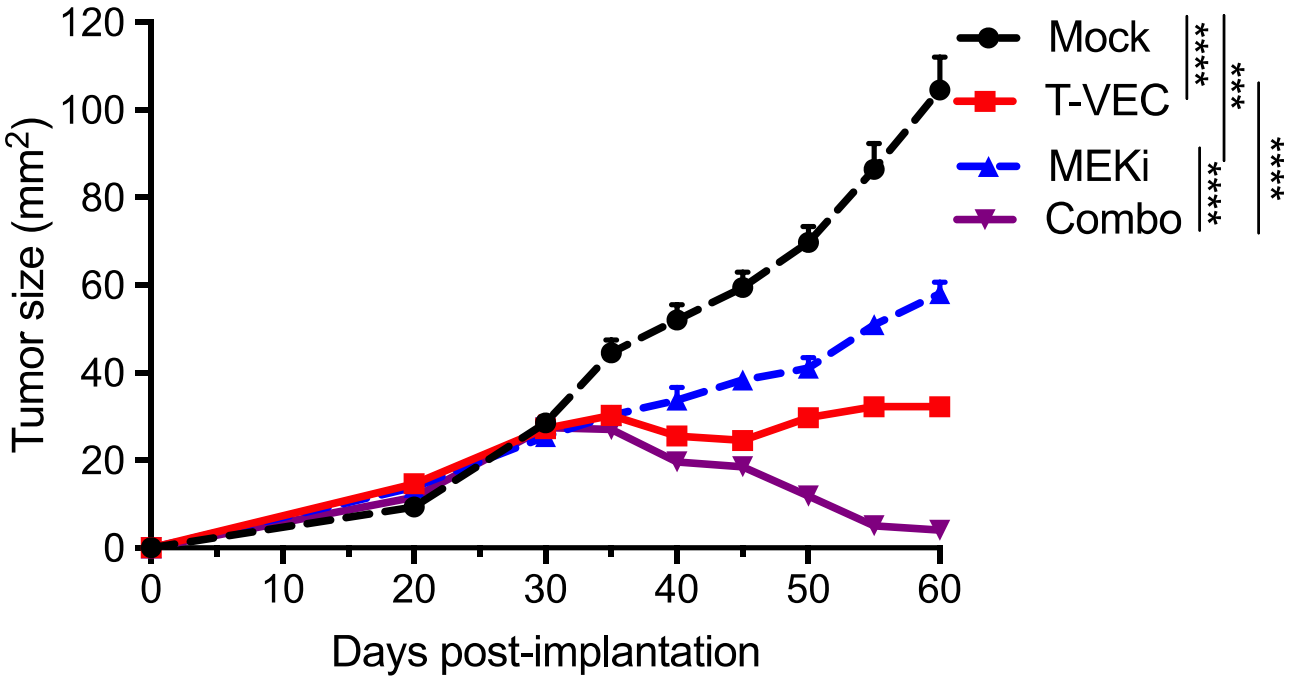
T-VEC + pembrolizumab induces CR in immunologically deserted tumors



Ribas et al. Cell 2017

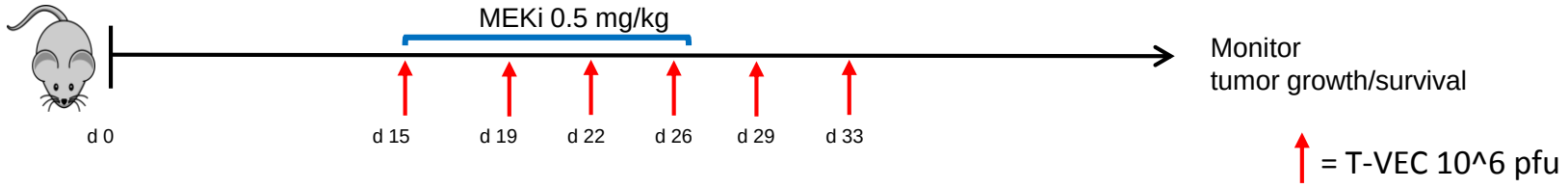
T-VEC and MEK inhibition promotes tumor regression in the SK-MEL-28 xenograft melanoma model



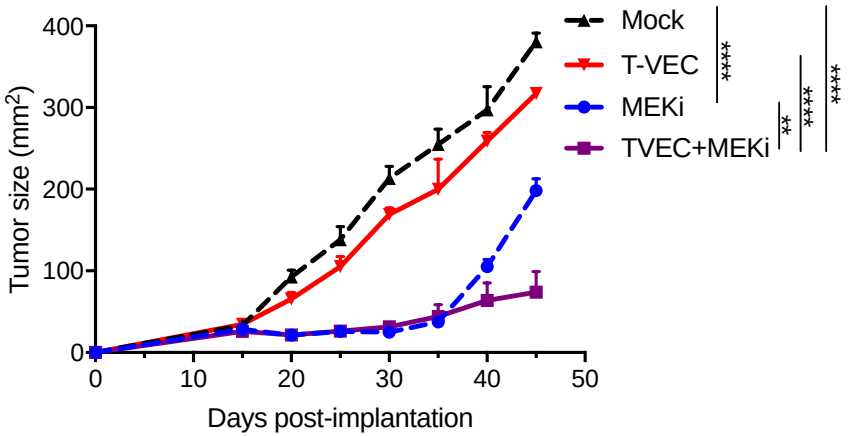


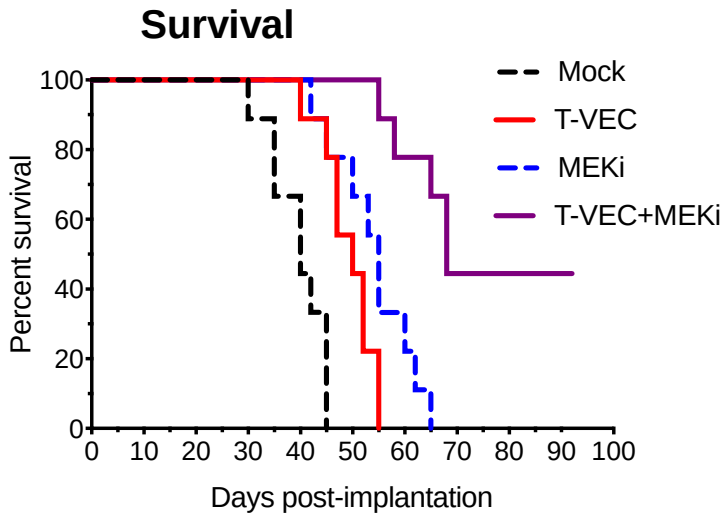
Bommareddy et al. Science Transl Med 2018

TVEC and MEKi reduces tumor growth in immune competent D4M3A melanoma model



n = 9

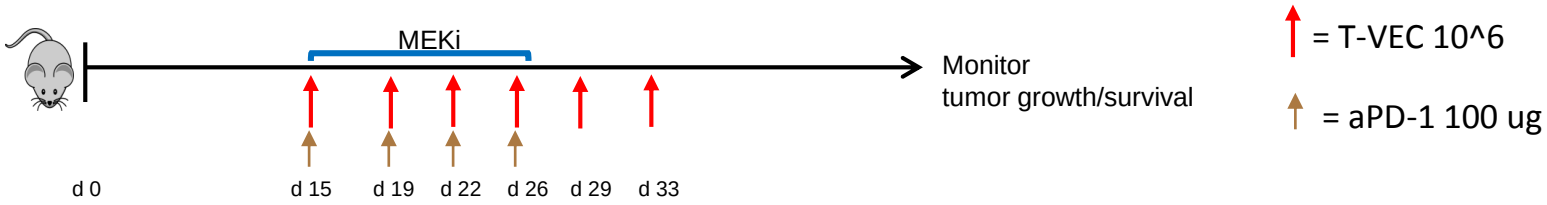


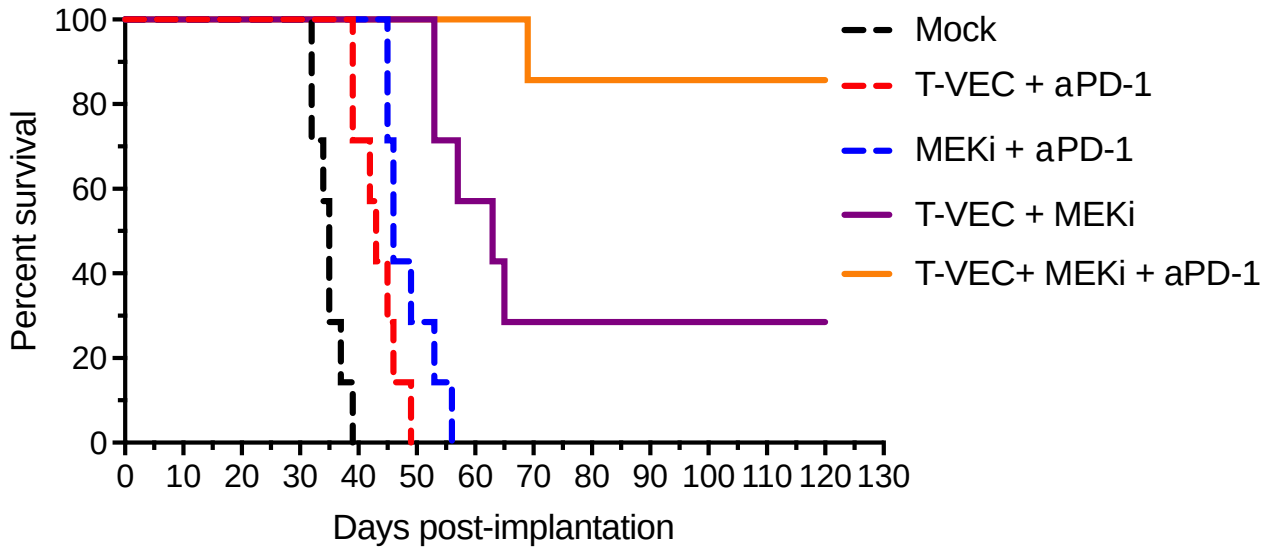


50% survival

Bommareddy et al, Sci Trans Med. 2018

PD-1 blockade augments T-VEC + MEKi combination treatment





90% survival

Bommareddy PK et al, Sci Trans Med. 2018

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?

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^{8}–3×10^{9} 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

T-VEC neoadjuvant study shows promise

- 150 stage IIIb-IVM1a melanoma patients with at least one resectable 1 cm tumor
- No systemic treatment in prior 3 months
- Randomized to T-VEC 6 doses over 12 weeks followed by surgery weeks 13-18 or up-front surgery
- Primary endpoint is RFS

- CR 22.8% (13 of 57) in efficacy analysis set (17% in ITT population)
- R0 resection rate 56.1% (32 of 57) in T-VEC arm vs. 40.6% (28 of 69) in surgery-only arm
- RFS improved in T-VEC group HR 0.73 [80% CI, 0.56-0.93; P=0.048]
- OS improved in T-VEC group 95.9% vs. 85.8% in surgery-only group [HR, 0.47; 80% CI, 0.27-0.82)
- Median f/o only 20 months

Dummer et al. ASCO 2019

Conclusions

- Intratumoral immunotherapy (ITIT) is defined as local delivery of agents that induce innate/adaptive anti-tumor immune responses
- There are many types of ITIT in clinical development
  - Physical approaches
  - Drug-based approaches
- ITIT has special 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
- ITIT has special clinical and logistical considerations
  - Must consider dosing, schedule, volume, biodistribution, anti-viral responses, eligibility and endpoint responses
- ITIT can be used as part of a rational combination approach
  - Neoadjuvant, IO combinations, non-IO combinations

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