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Society for Immunotherapy of Cancer

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Toll Like Receptors 7/8 in Cancer Therapy

Willem Overwijk, PhD

NEKTAR Therapeutics

Presenter Disclosure Information

Willem Overwijk, PhD
Nektar Therapeutics

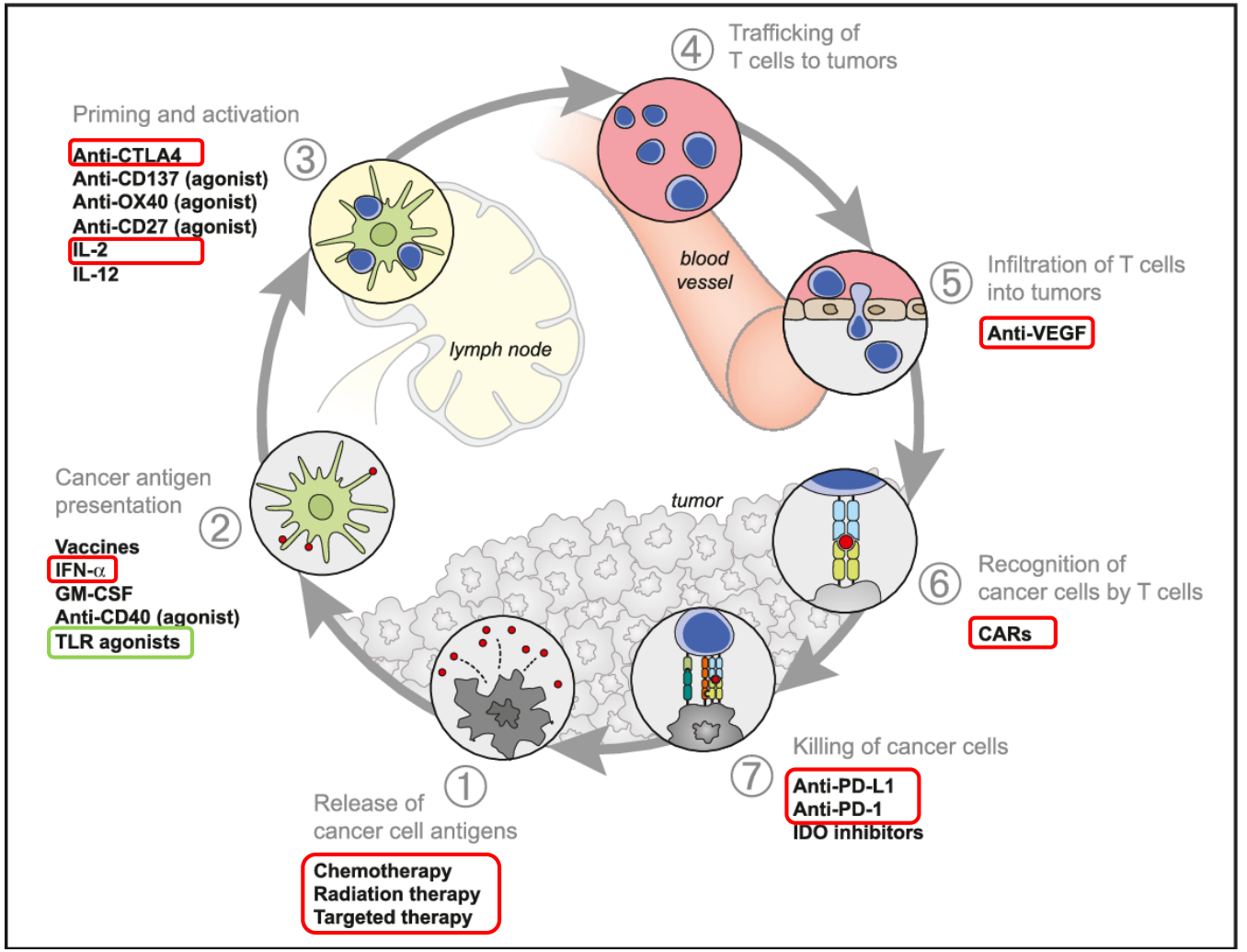
The following relationships exist related to this presenter:

- Employee and Shareholder of Nektar Therapeutics
- Nektar Therapeutics develops the intratumoral TLR7/8 agonist, NKTR-262

A Place for TLR Agonists in the Cancer Immunity Cycle

<u>Ideal Scenario:</u>

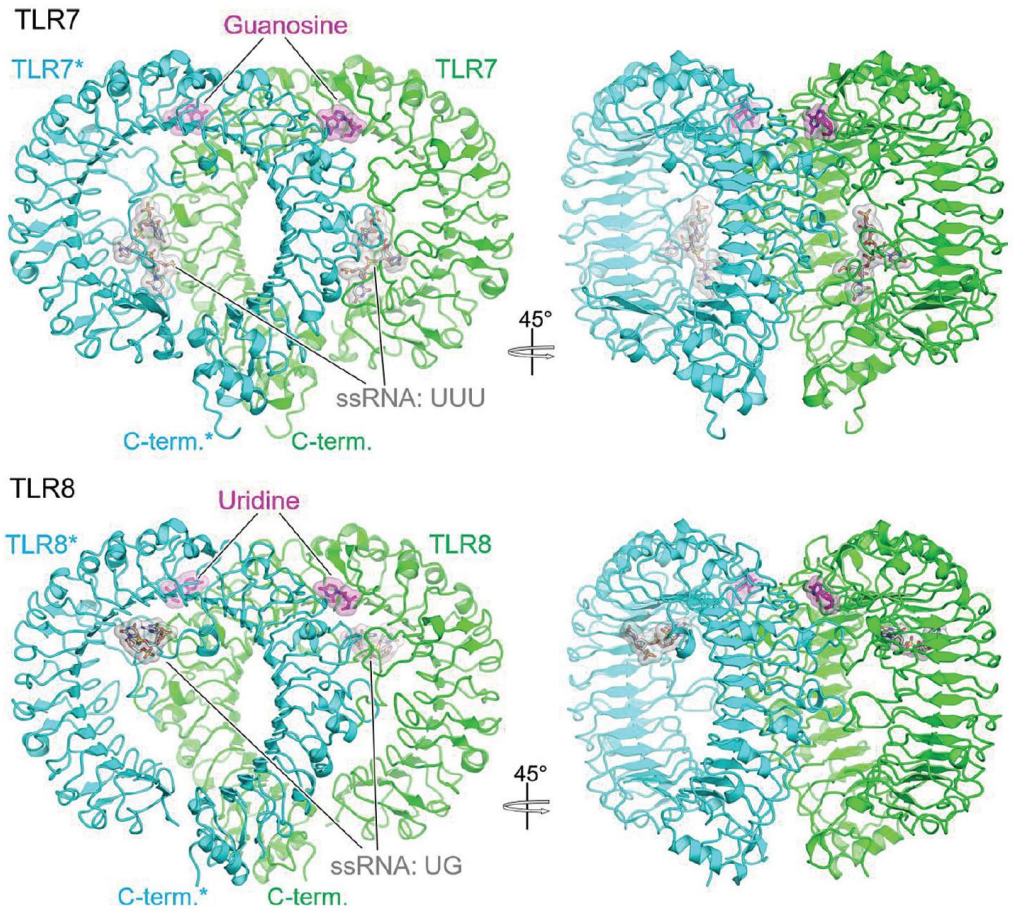
1. Intratumoral presence of TLR agonist
2. Antigen presenting cell maturation
3. Tumor antigen presentation to T cells
4. T cell (re-)activation
5. Systemic T cell response
6. Systemic tumor eradication



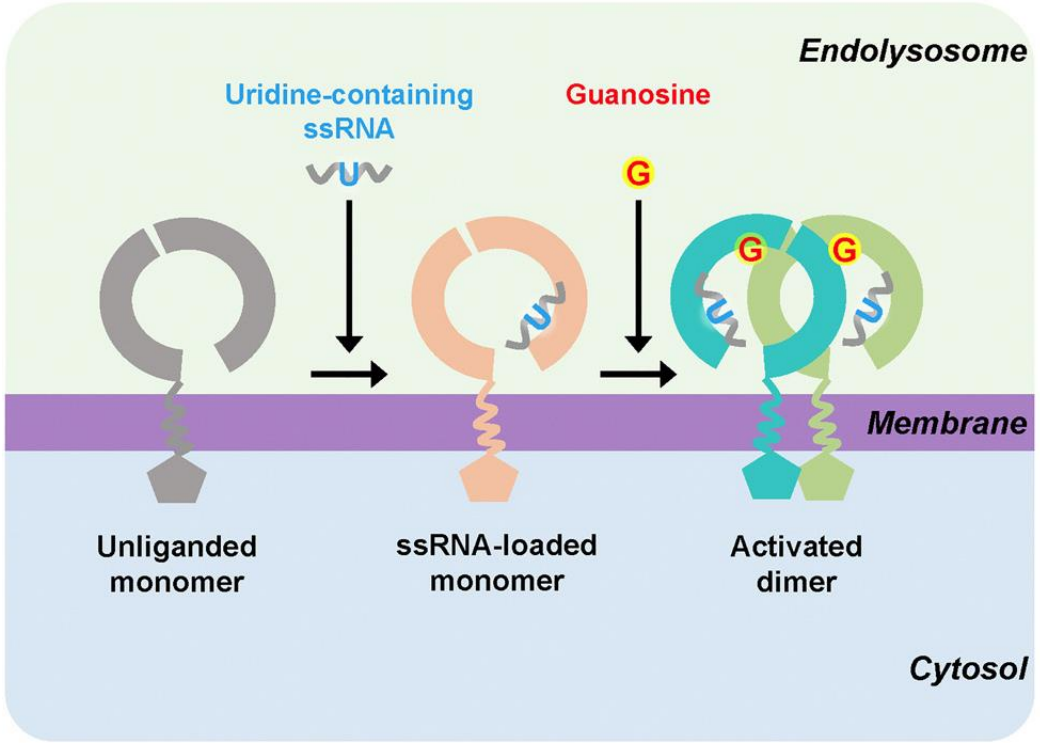
Chen and Mellman. *Immunity.* 2013;39:1-10.

TLR7 and TLR8 Structure and Signaling

TLR 7/8 Molecular Structure and Natural Ligand Binding Sites

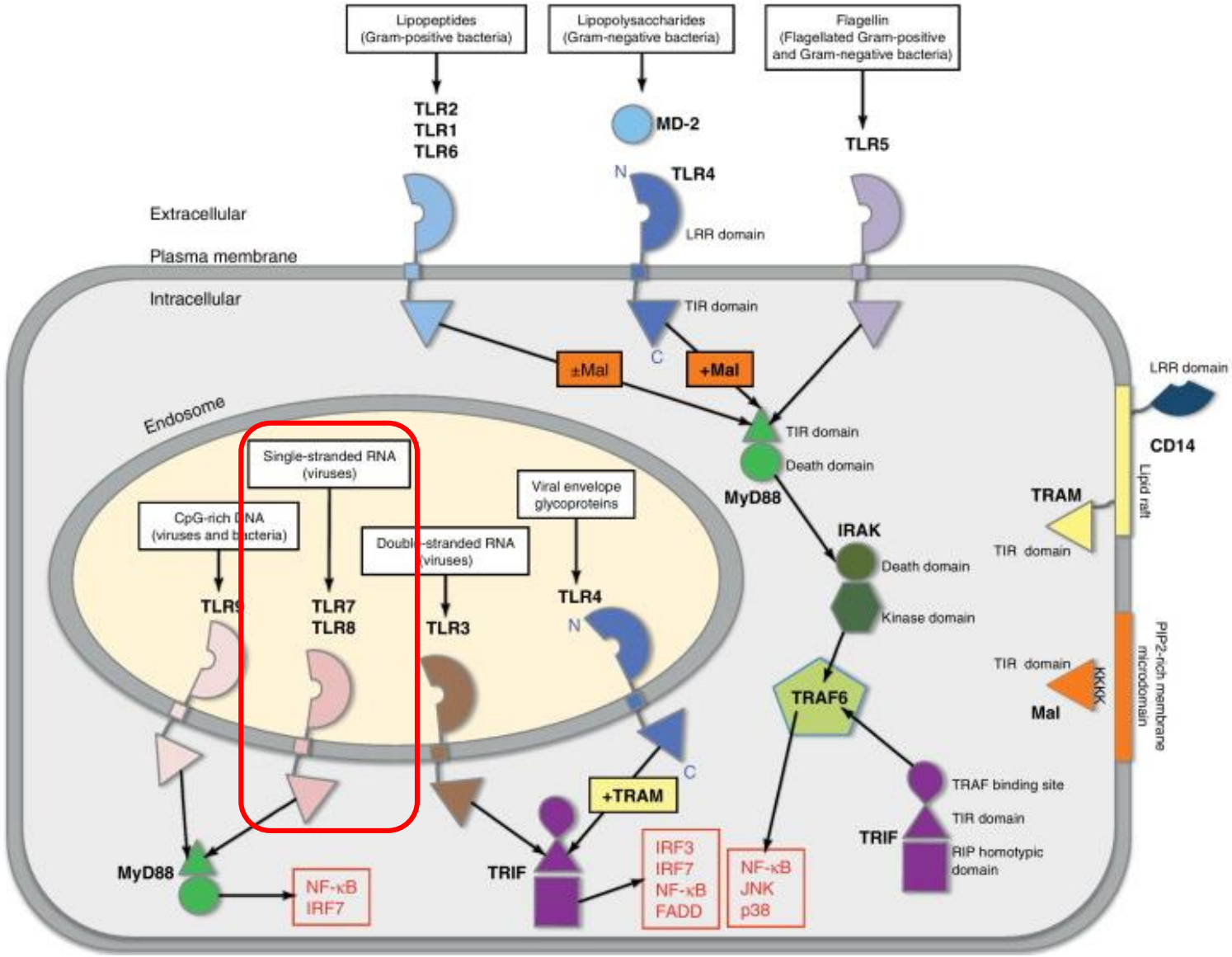


TLR7/8 ligands: ssRNA and ssRNA-derived guanosine (G) and uridine (U)



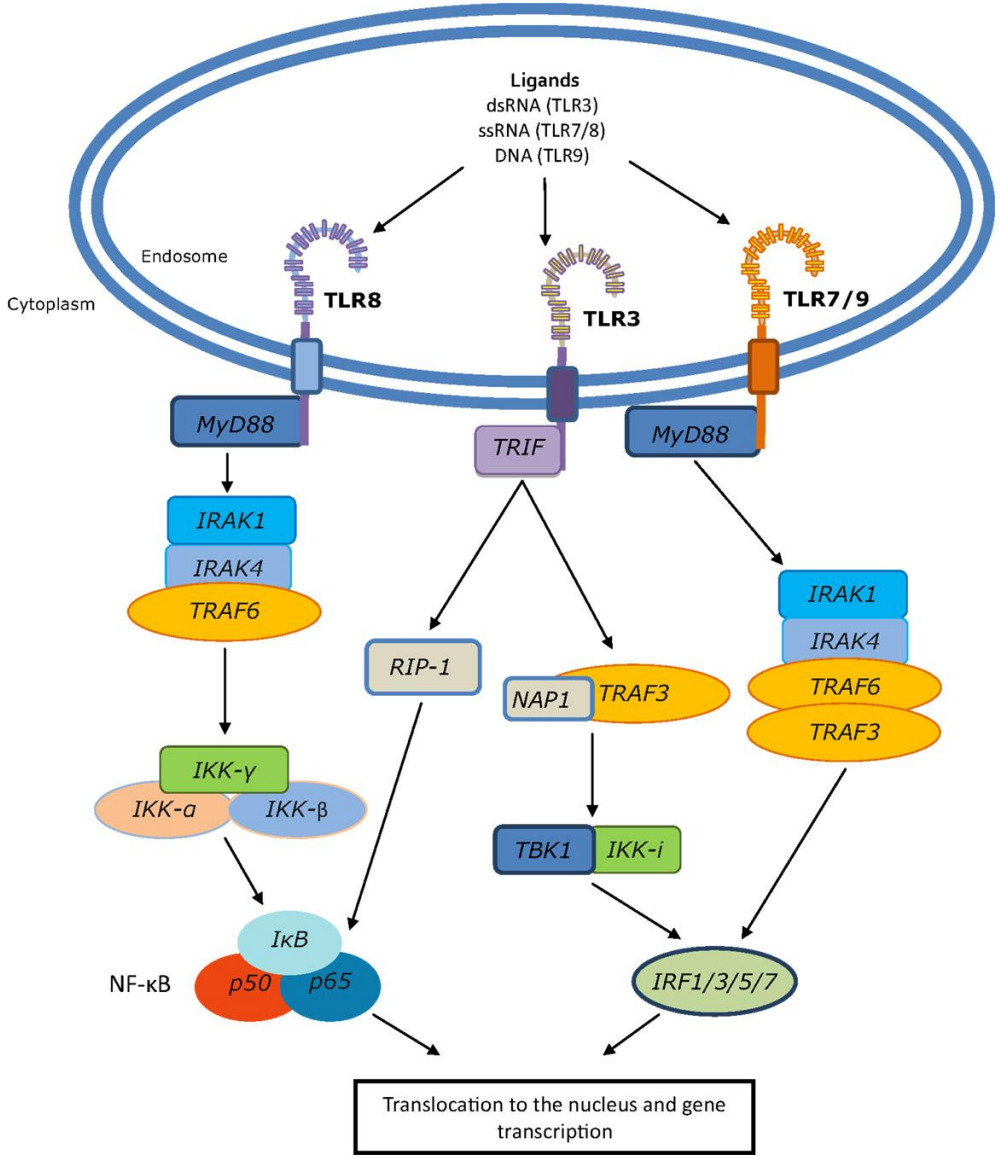
Miyake *et al.*, Immunity 2016

TLR7/8 Are Expressed in Endosomes



Gangloff. *Trends Biochem Sci*. 2012;37:92-8.

Consequence of TLR7 *vs.* TLR8 Signaling



Thwaites et al. *Front Immunol*. 2014 Jan 16;5:1. doi: 10.3389/fimmu.2014.00001.

Expression of TLR 7/8 and Consequences of Triggering

Table 1 TLR expression and functionality on DC subtypes

DC subtype	TLR	Expression	Effects of activation
moDC	1	+	See TLR2
	2	++	Increased IL-6, IL-8, IL-10, IL-12, TNFα
			Low IFNβ response and no IFNα response
	3	+	Specific IFNβ mRNA upregulation (not IFNα)
	4	++	Upregulation of CD80, CD86, CD83, CCR7
			Secretion of IFNβ, IFNγ, IL-1β, IL-12p70, IL-13, IP-10
			Decreased endocytic capacity
	5	+	Upregulation of CD80, CD86, CD83, CCR7
			Secretion of IFNγ, IL-1β, TNF, IL-8, IL-12p40 (not IL-12p70), IL-13
			Decreased endocytic capacity
	6	±	See TLR2
	7	±	–
	8	+	Increased TNFα, IL-8, IL-12p40, MCP-1, CCL2, CCL3, CCL4, CCL5
	9	–	
	10	–	
mDC	1	+	See TLR2
	2	++	Upregulation of CCR7, IL-6, IL-10, IL-12p70,TNFα, no INFα
	3	++	IFNα (intermediate), IL-12p70 (high)
			No TNFα or IL-6
	4	+	Upregulation of CD80, CD86, CD83, CD40, CCR7
			Secretion or upregulation of CCR7, IL-6, IL-8, IL-10, IL-12p70
			No IFNα response
	5	+	Upregulation of CD80, CD86, CD83, CCR7
			Secretion of TNF and IL-8
			Upregulation of CCR7
	6	+	See TLR2
	7	+	Upregulation of CD40, CD80 and CD86
			Secretion of IL-12p70
			No IFNα response
	8	±	See TLR7
	9	–	
	10	+	No ligand known

DC subtype	TLR	Expression	Effects of activation
pDC	1	±	
	2	–	
	3	–	
	4	–	
	5	–	
	6	–	
	7	++	Upregulation of CD40, CD80, CD86, CCR7
			Very high IFNα response
			No IL-12p70 response
	8	–	
	9	+++	Upregulation of CD40, CD80, CD86, CD83, HLA-DR, CCR7
			Upregulation of IFNα (very high), IFNβ (lower), IL-6, TNFα (low), IL-8, IP-10
			No IL-10 secretion
	10	+	No ligand known

Schreibelt et al. *Cancer Immunol Immunother*. 2010;59:1573-82.

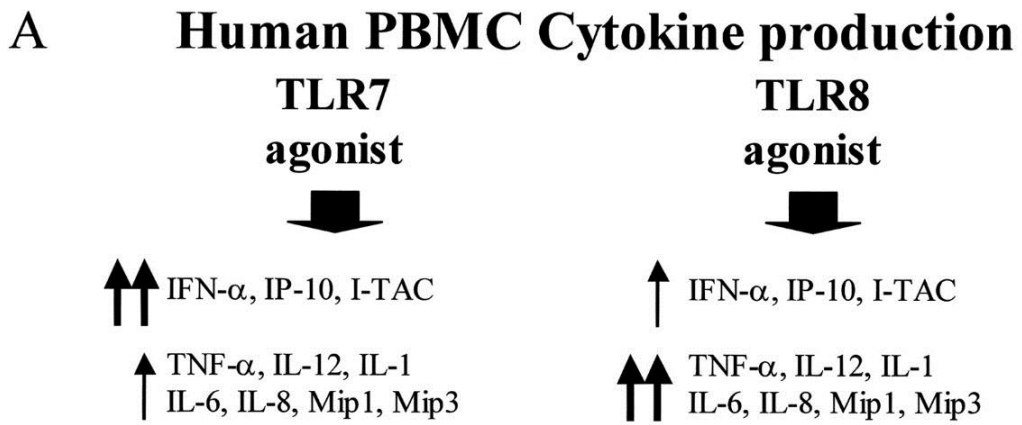
Expression of TLR 7/8 and Consequences of Triggering

Table 1 Phenotype and function of human DC subsets

	CD1c$^+$ DC	CD141$^+$ DC	pDC	MoDC	LC
Mouse equivalent	CD11b$^+$ cDC	CD8$^+$ cDC	pDC	Inf DC	LC
Location	Blood, lymphoid, non-lymphoid organs	Blood, lymphoid, non-lymphoid organs	Blood, lymphoid organs	Skin (steady state), inflamed tissues (inflammation)	Skin
Phenotype	Lin$^-$ CD11c$^+$ DR$^+$ Sirpα (CD172a)$^+$ CD1c$^+$ CD14^{low} CD11b$^+$ CD103$^+$ (intestine only)	Lin$^-$ CD11c$^+$ DR$^+$ CD141$^+$ XCR1$^+$ CLEC9A$^+$ Necl2$^+$	Lin$^-$ CD11c$^-$ DR$^+$ BDCA-2$^+$ BDCA-4$^+$ CD123$^+$	Lin$^-$ CD11c$^+$ DR$^+$ CD1a$^+$ CD1c$^+$ CD14$^+$ CD11b$^+$ FcεRI	Lin$^-$ CD11c$^+$ DR$^+$ CD1c$^+$ Langerin$^+$ E-cadherin$^+$
TLR expression	3 (low), 4 (low), 8	3, 8	7, 9	3 (low), 4, 7 (low), 8	3
Cytokine profile	IL-12, IL-23, IL-10	Type III	Type I and III IFN	IL-1β, IL-6, IL-10, IL-23	ND
Functions to date	Th2 induction in response to allergen, Th17 induction in response to fungal infection, immune regulation	Cross presentation of cellular Ag and immune complexes, CTL priming.	Type I IFN production against viral and fungal infections, immune regulation	Induction of Th1 and Th17 responses	Induction of Th2, CTL responses

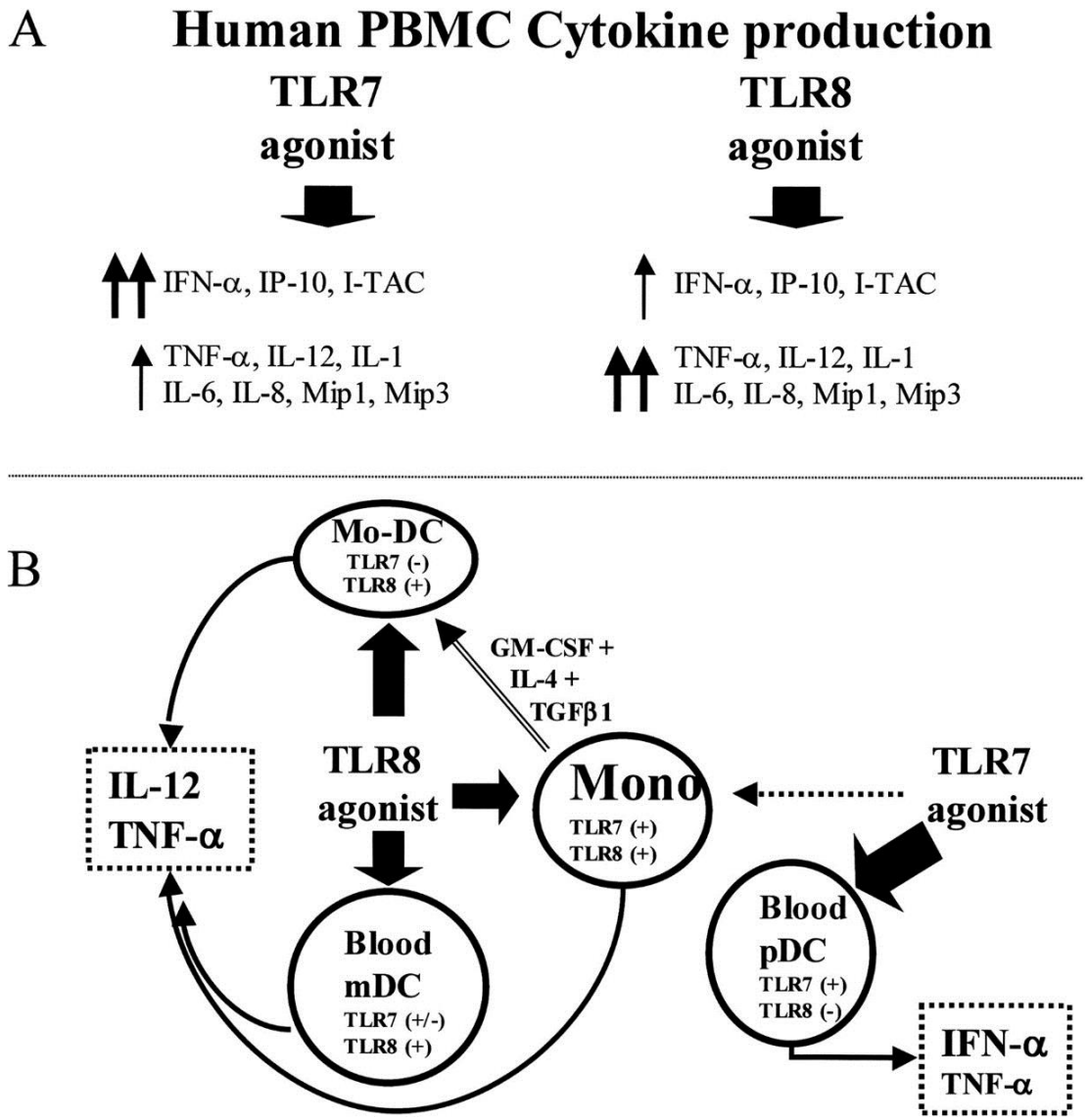
O'Keeffe et al. *Cell Mol Life Sci*. 2015;72:4309-25.

TLR7 vs TLR8 Response in Whole PBMCs



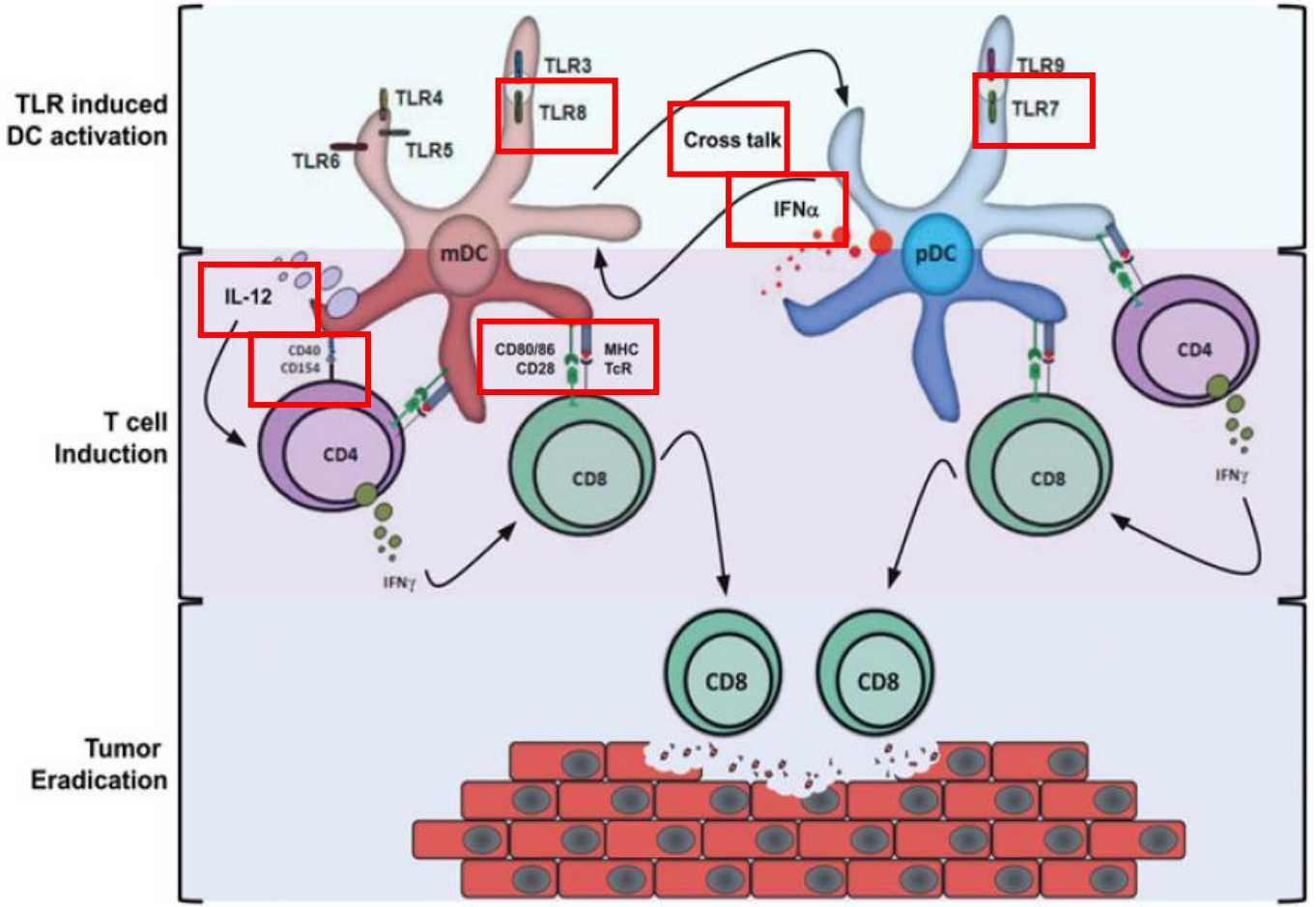
Keith B. Gorden et al. *J Immunol* 2005;174:1259-1268

TLR7 vs TLR8 Response in Whole PBMCs



Keith B. Gorden et al. *J Immunol* 2005;174:1259-1268

TLR7 on pDCs and TLR8 on mDCs: Consequences



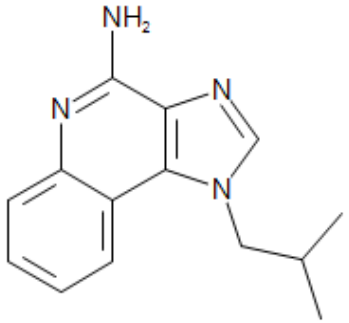
Schreibelt et al. *Cancer Immunol Immunother*. 2010;59:1573-82.

Preclinical Studies:

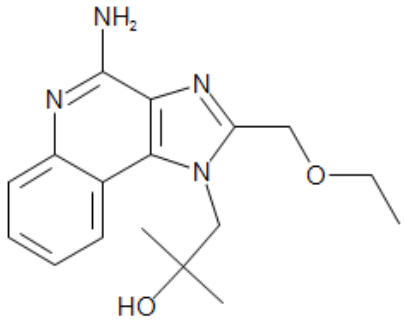
MOA and Potential for Combinations

Structural Similarity Among Some Imidazoquinoline Compounds Under Investigation

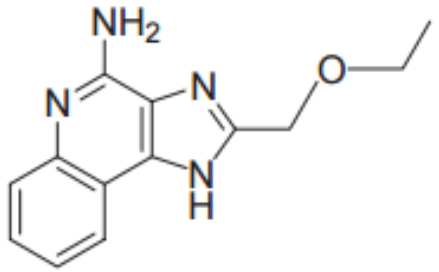
Imiquimod (TLR7)



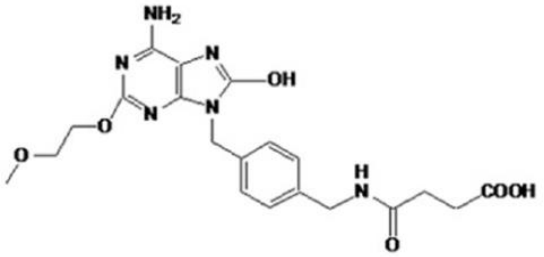
Resiquimod/R848 (TLR7/8)



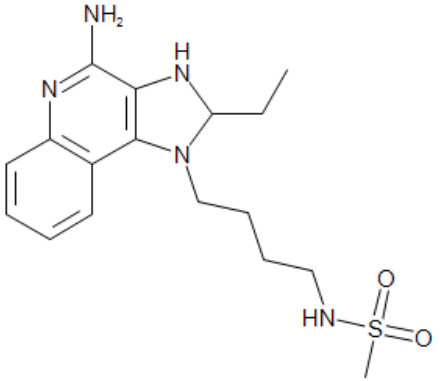
CL097 (TLR7/8)



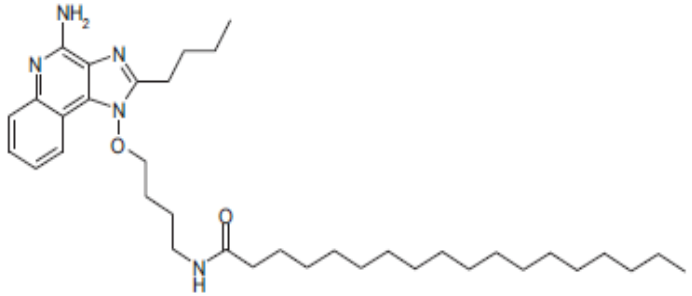
SZU-101 (TLR7)



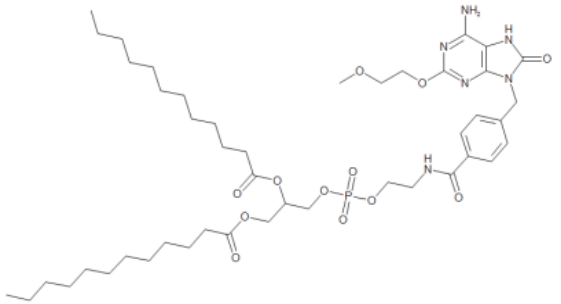
852A (TLR7)

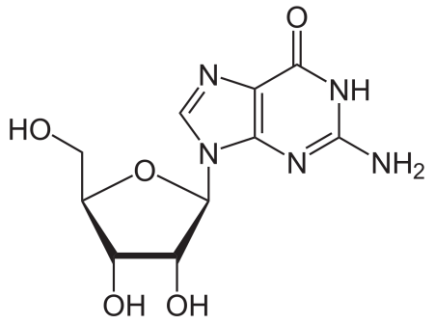


MEDI9197/3M-052 (TLR7/8)



TMX-202 (TLR7)





Guanosine (TLR7)

Preclinical and Clinical Oncology Studies with TLR7/8 Agonists

	Agonist	Tumor type	*In vitro*	*In vivo*	References
TLR7	Imiquimod	Squamous carcinoma	YS-10B, FaDu,	/	Ahn et al., 2012
		Prostate cancer	TRAMP-C2, PC3	C57BL/6	Han et al., 2013
		Bladder cancer	MB49	C57BL/6	Hayashi et al., 2010
		Breast cancer	TSA	BALB/c Human (preclinical)	Adams et al., 2012; Dewan et al., 2012
		Melanoma	/	Human (preclinical)	Narayan et al., 2012
TLR7/8	Resiquimod	Gliomas	GL261	C57BL/6	Grauer et al., 2008
		Acute myeloid leukemia	HL60, THP1, OCI-AML3, HCT116, 293T	Nod/SCID/IL2Rγ-/-(NSG)	Smits et al., 2010; Ignatz-Hoover et al., 2015
		Breast cancer	4T1	BALB/c	Yin et al., 2015
		T-cell lymphoma	/	Human (phase I)	Rook et al., 2015
TLR7	Gardiquimod	Melanoma	B16	C57BL/6	Ma et al., 2010
		Pancreatic cancer	BxPC-3	/	Zou et al., 2015
TLR7	852A	Ovarian cancers	/	Human (preclinical)	Geller et al., 2010
		Cervix cancer	/	Human (preclinical)	Geller et al., 2010
		Breast cancer	/	Human (preclinical)	Geller et al., 2010
		Melanoma	/	Human (phase II)	Dummer et al., 2008
		Lymphocytic leukemia	/	Human (phase I/II)	Spaner et al., 2010
TLR7	Loxoribine	Melanoma	B16	C57BL/6	Pope et al., 1994
		B-chronic leukemia	/	Human (preclinical)	Tosi et al., 1997; Pellacani et al., 1999
TLR7	Bropirimine	Bladder tumor	KK-47 724	/	Tei et al., 2002
		Prostate cancer	MBT-2	/	Sarosdy, 1997
		Renal-cell carcinoma	Renca	BALB/c	Fujioka et al., 1995
TLR7/8	3M-011	Pancreatic cancer	BxPC3 Panc-1	BALB/c C57BI/6	Scholch et al., 2015
		Colon cancer	HT29 HCT-116	BALB/c C57BI/6	Scholch et al., 2015

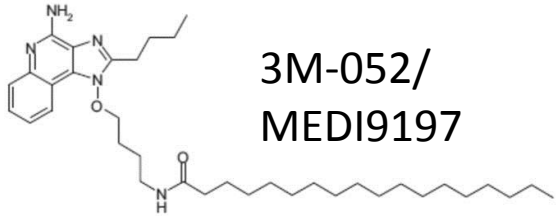
Chi et al., Front. Pharm. 2017

Preclinical and Clinical Oncology Studies with TLR7/8 Agonists

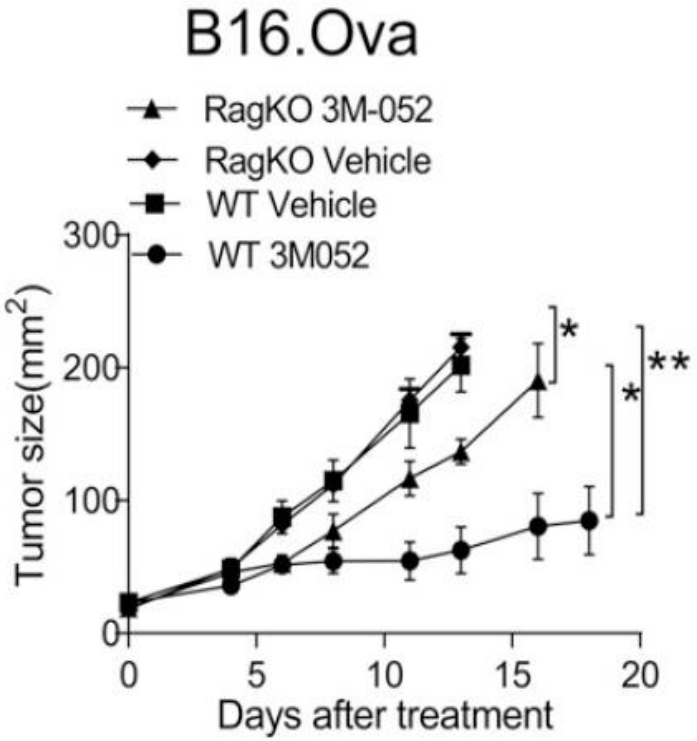
	Agonist	Tumor type	*In vitro*	*In vivo*	References
TLR7/8	3M-052	Melanoma	B16.F10, B16.OVA, BP	C57BL/6	Singh et al., 2014
TLR7	DSR-6434	Colon cancer Renal cell carcinoma	CT26 Renca	C3H BALB/c Balb/c C57BL/6	Adlard et al., 2014 Koga-Yamakawa et al., 2015
TLR7	DSR-29133	Colon cancer Osteosarcoma Renal cell carcinoma	CT26 LM8 Renca	Balb/c C3H Balb/c	Dovedi et al., 2016 Dovedi et al., 2016 Dovedi et al., 2016
TLR7	SC1	Lymphoma Renal cell carcinoma	RMA-S Renca	C57BL/6 Balb/c	Wiedemann et al., 2016 Hamm et al., 2009
TLR7	SZU-101	Breast carcinoma Gastric cancer T cell lymphoma	4T1 EAC EL4	Balb/c Balb/c C57BL/6	Diao et al., 2016 Wang et al., 2015 Zhu et al., 2015
TLR7	SM-360320	Colon cancer	MC38	BALB/c	Dharmapuri et al., 2009
TLR7	SM-276001	Renal cell carcinoma Colon cancer	Renca CT26	Balb/c Balb/c	Koga-Yamakawa et al., 2013 Koga-Yamakawa et al., 2013
TLR7/8	Resiquimod	Gliomas Acute myeloid leukemia Breast cancer T-cell lymphoma	GL261 HL60, THP1, OCI-AML3, HCT116, 293T 4T1 /	C57BL/6 Nod/SCID/IL2Rγ-/-(NSG) BALB/c Human (phase I)	Grauer et al., 2008 Smits et al., 2010; Ignatz-Hoover et al., 2015 Yin et al., 2015 Rook et al., 2015
TLR8	VTX-2337	Lymphoma	/	Human (phase I)	Northfelt et al., 2014
TLR7/8	3M-011	Pancreatic cancer Colon cancer	BxPC3 Panc-1 HT29 HCT-116	BALB/c C57BI/6 BALB/c C57BI/6	Scholch et al., 2015 Scholch et al., 2015

Chi et al., Front. Pharm. 2017

TLR7/8 Agonist Efficacy Depends on CD8$^+$ T cells …

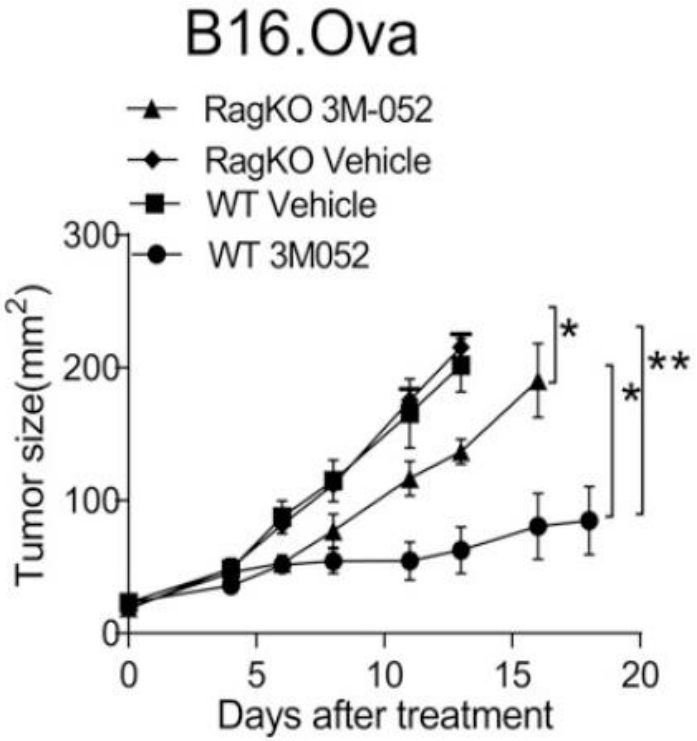


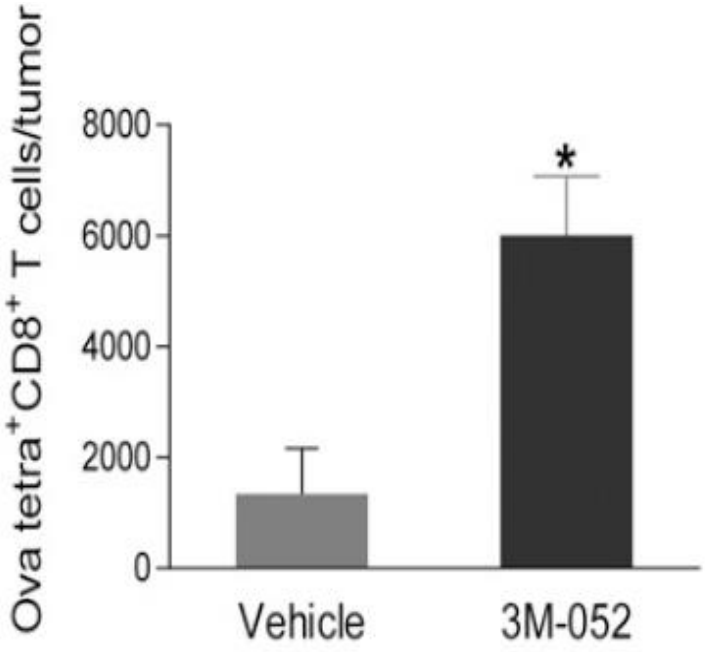
3M-052/
MEDI9197



Singh et al., *J. Immunol*. 2014

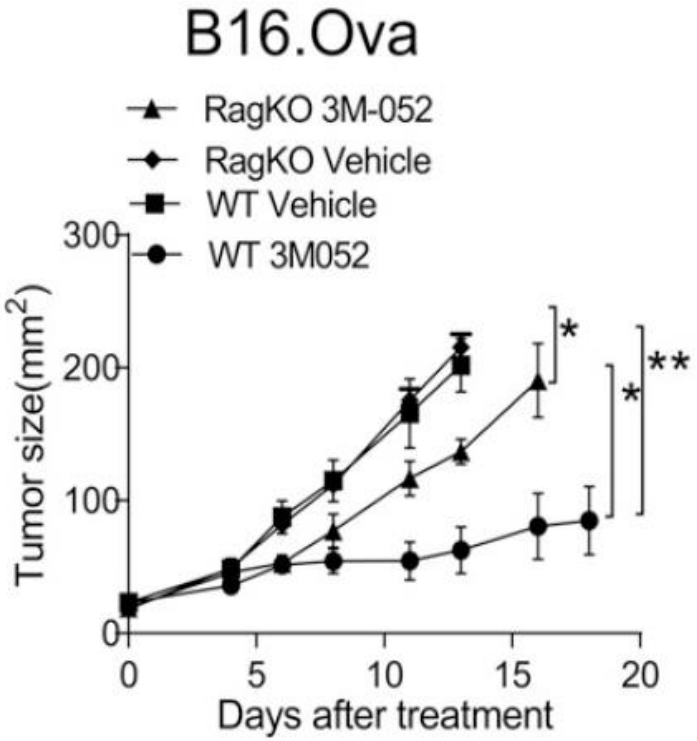
TLR7/8 Agonist Efficacy Depends on CD8$^+$ T cells …

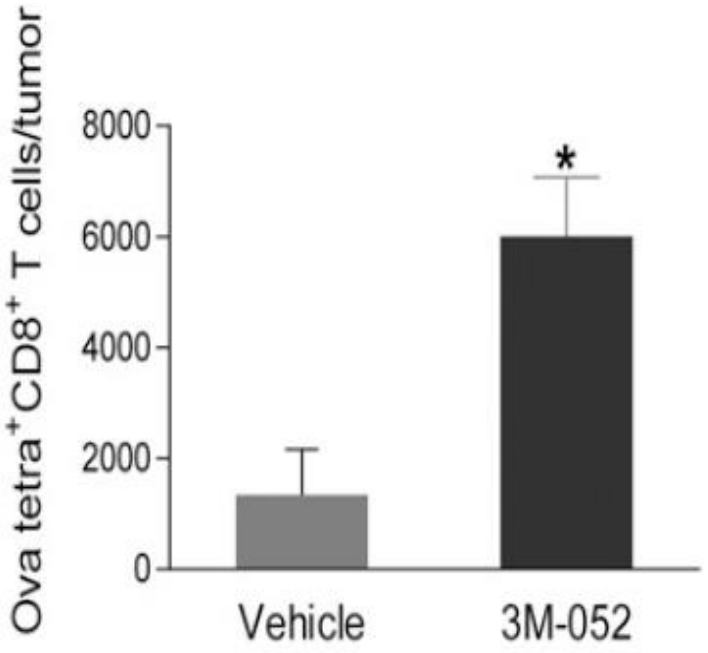


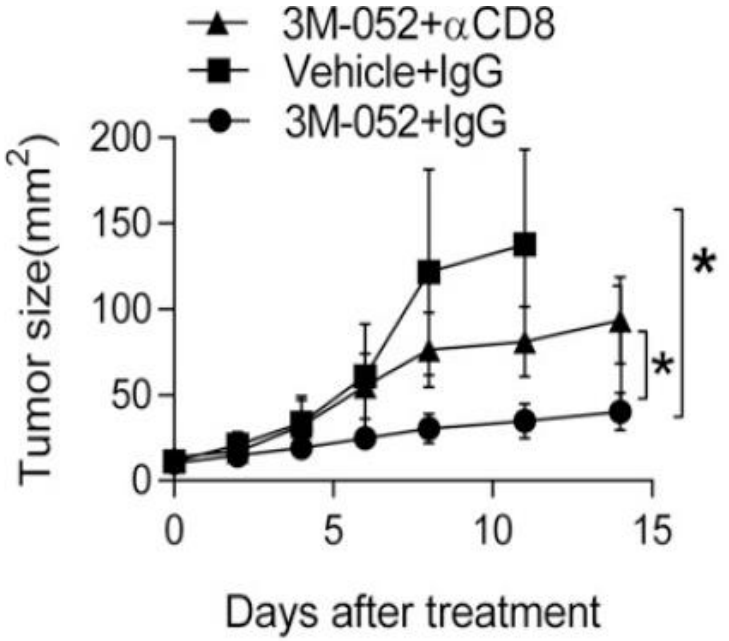


Singh et al., *J. Immunol*. 2014

TLR7/8 Agonist Efficacy Depends on CD8$^+$ T cells …



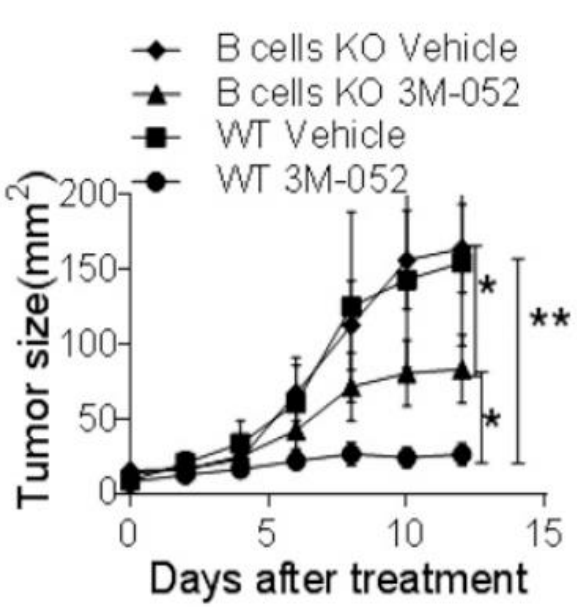




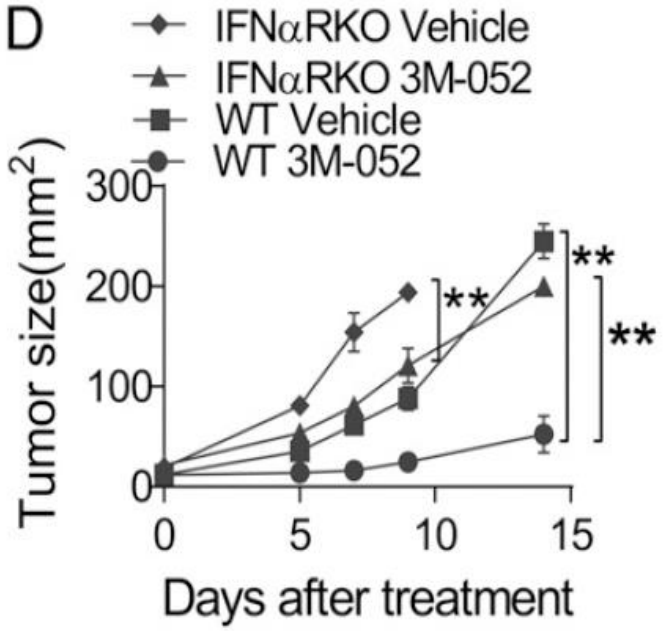
Singh et al., *J. Immunol*. 2014

… and Others!

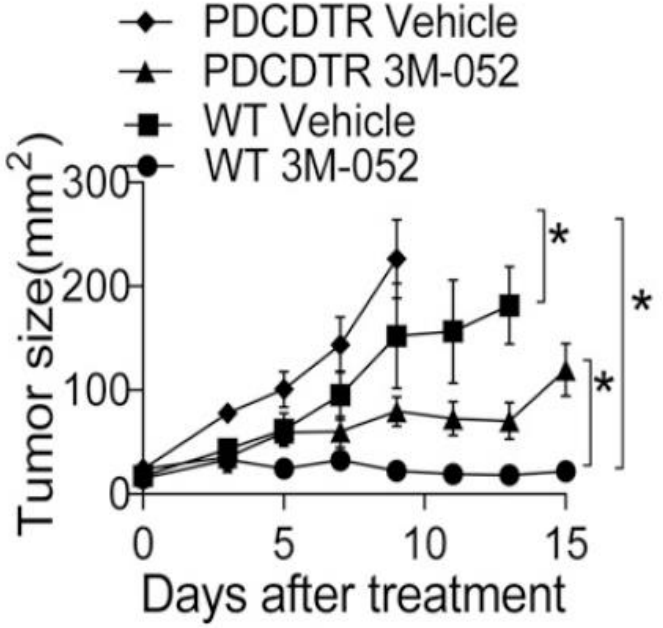
B cells



Type I IFN



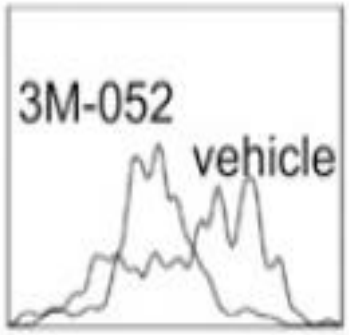
pDCs



But not $CD4^+$ T cells and NK cells

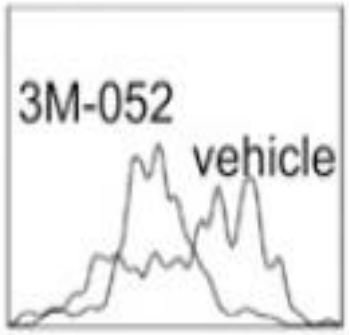
Singh et al., *J. Immunol*. 2014

Shift from Intratumoral M2 → M1 Macrophages



Singh et al., *J. Immunol*. 2014

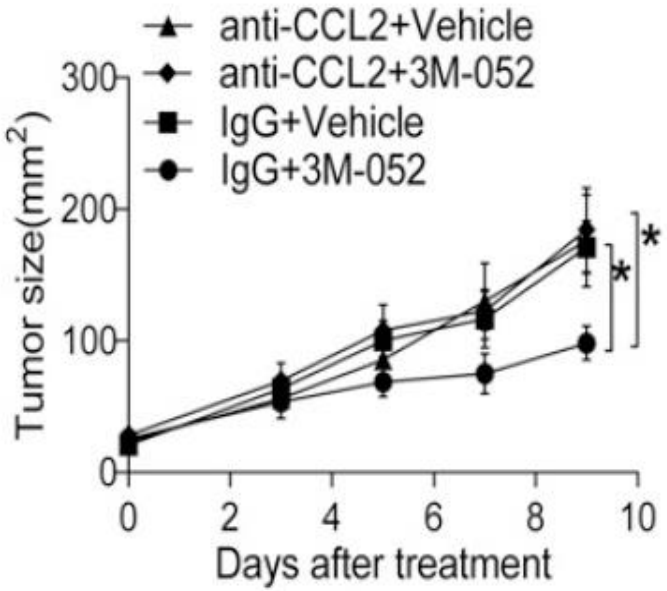
Shift from Intratumoral M2 → M1 Macrophages



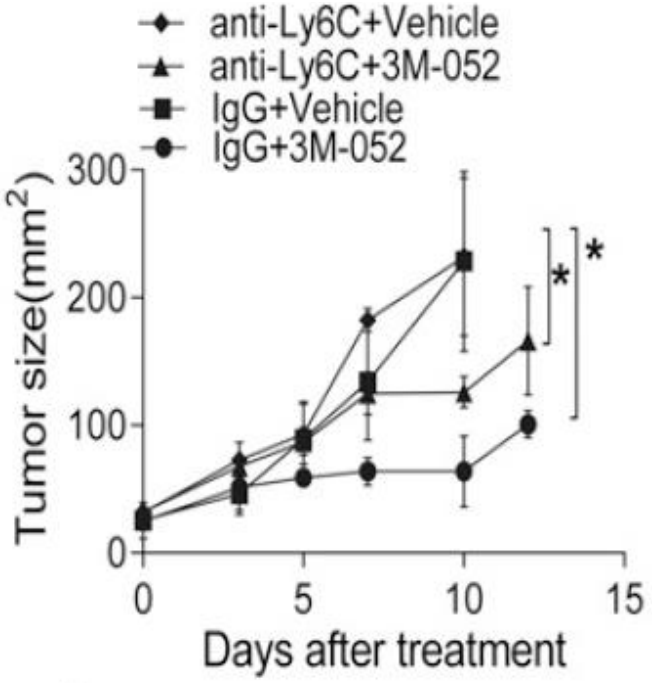
CD206 MFI
(Vehicle *VS* 3M-052)

1987±774
VS
659±156
P=0.04

MAC reduction method 1

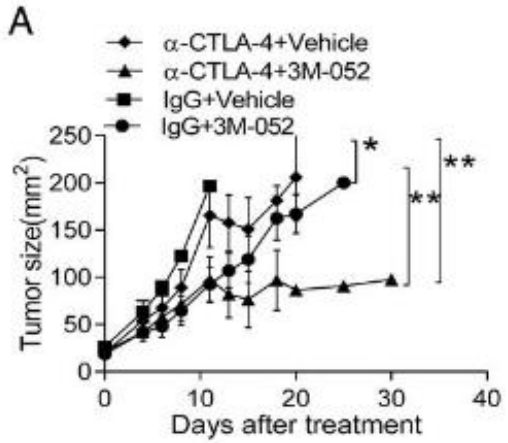


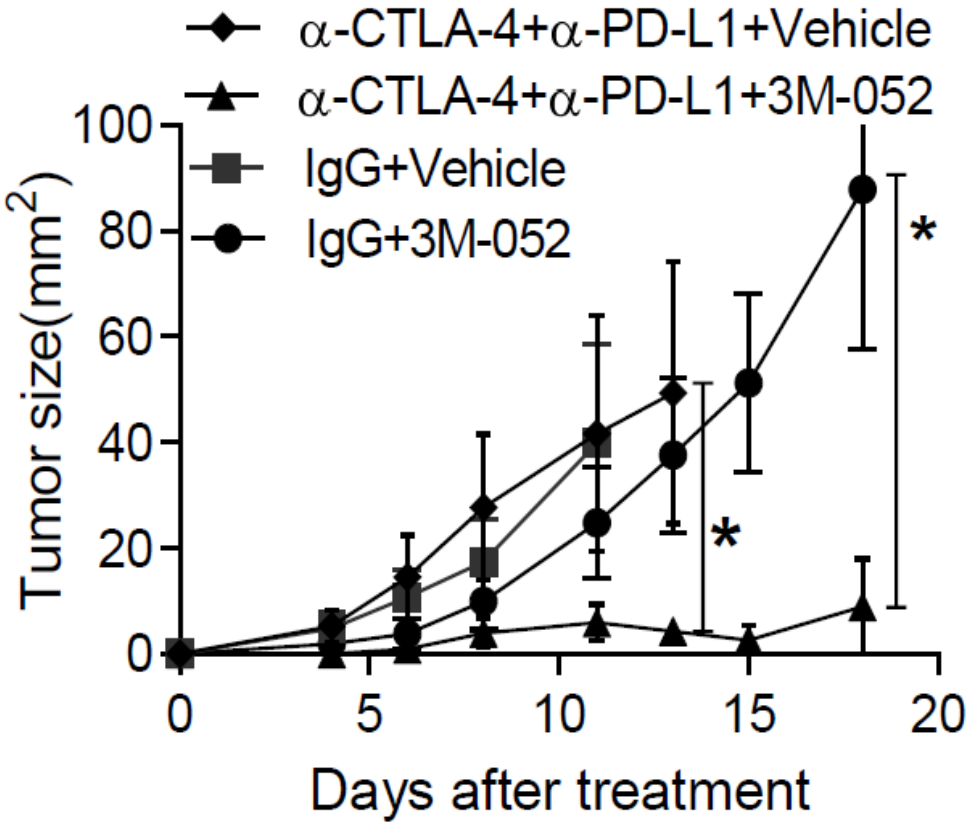
MAC reduction method 2



Singh et al., *J. Immunol*. 2014

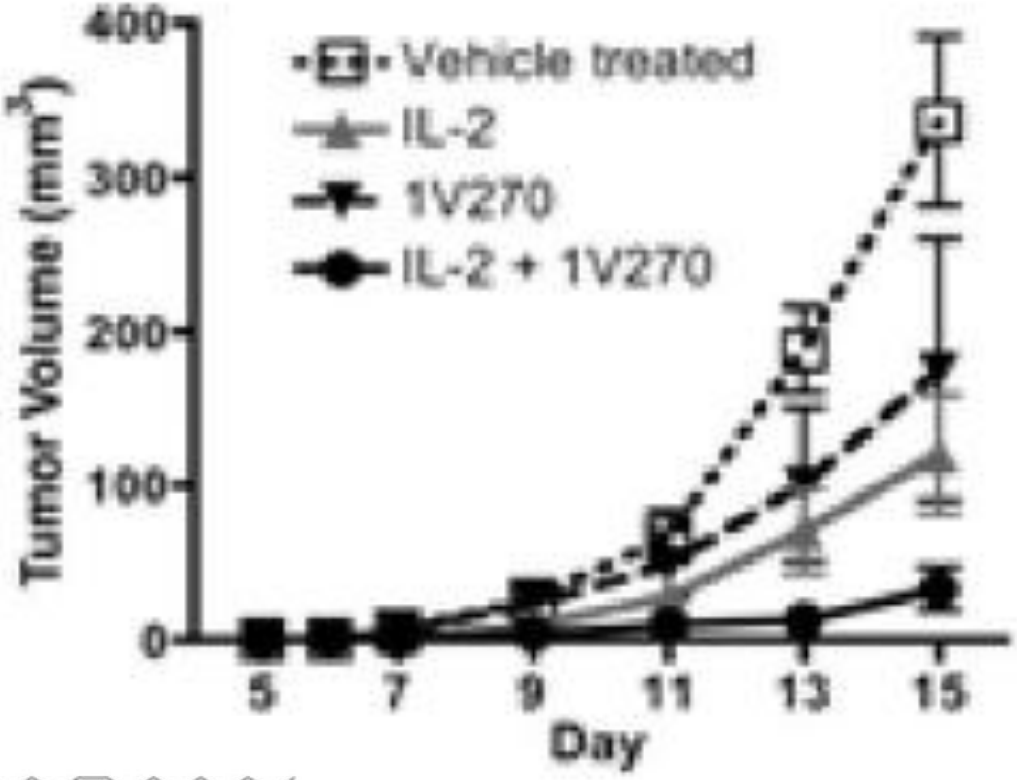
Combination of CTLA-4 and PD-L1 Checkpoint Inhibitors with Intratumoral TLR7/8 Agonist

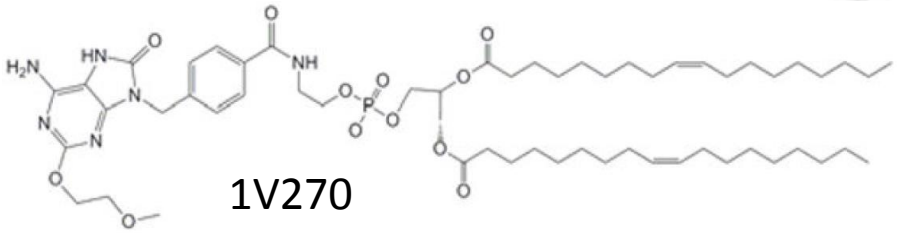




Singh et al., *J. Immunol*. 2014

Synergy Between IL-2 and Tumor-retained TLR7 Agonist

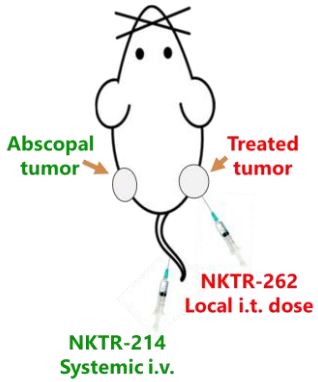


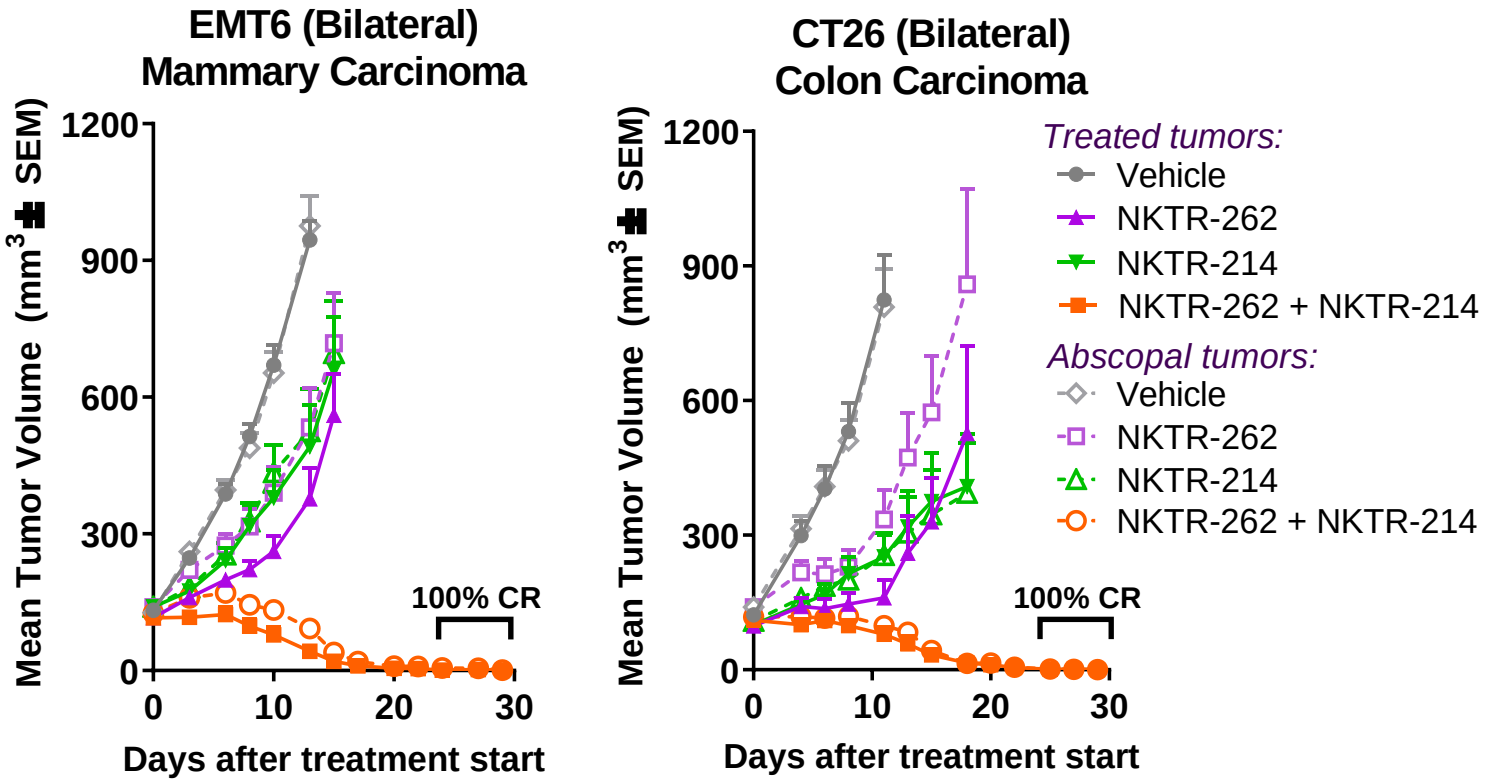


1V270

Hayashi *et al.,* Melanoma Res. (2011)

Combination Therapy with Tumor-Retained, PEGylated TLR7/8 Agonist and IL-2 Prodrug

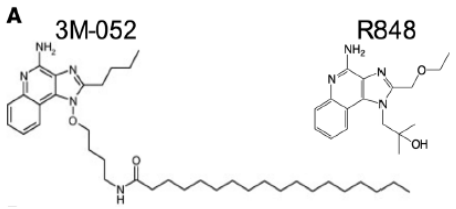


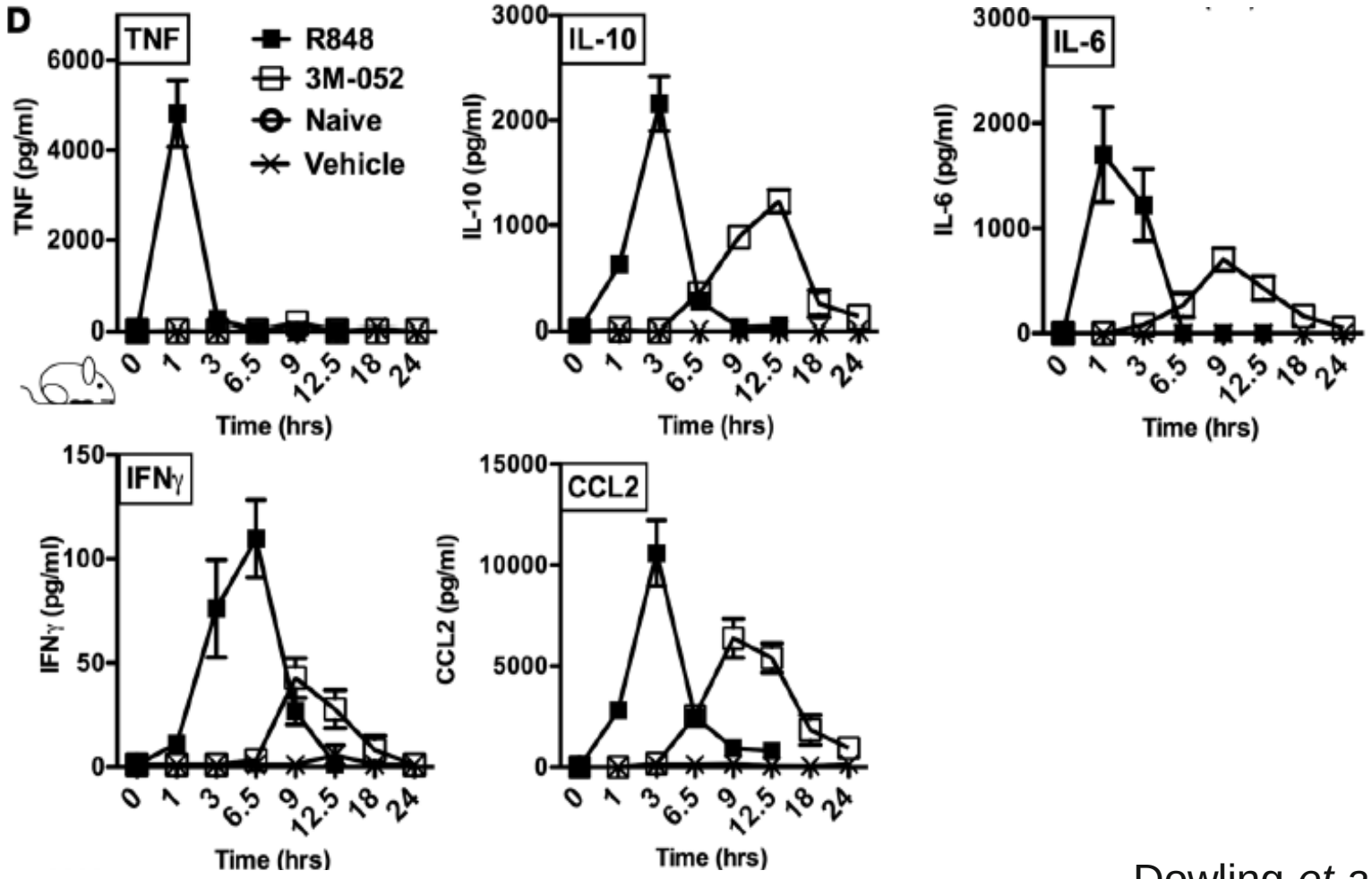


Kivimae *et al.*, SITC 2017

Why go Intratumoral and not Systemic?

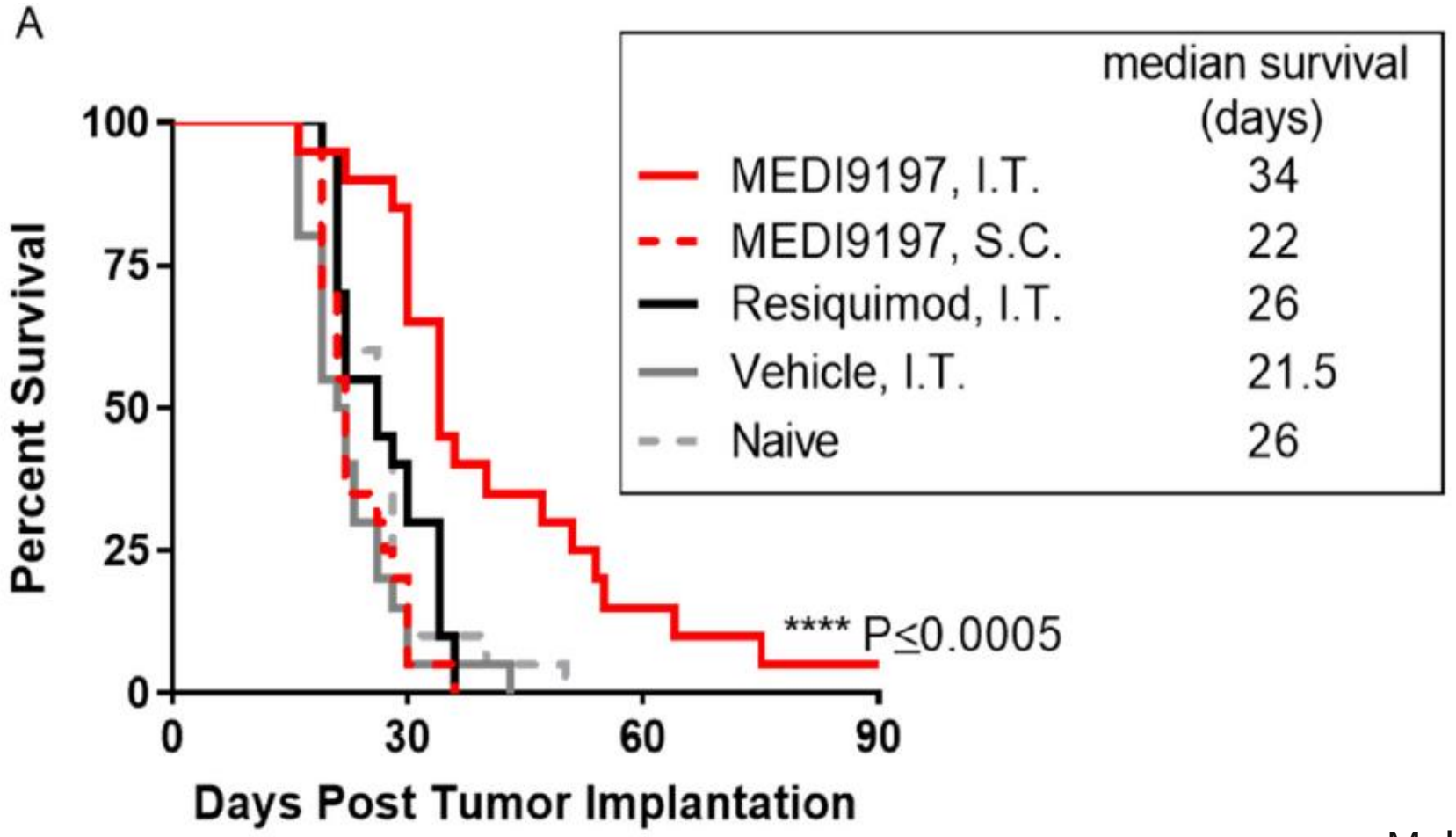
Tissue-retained TLR7/8 Agonist: Increased Tolerability





Dowling *et al.* JCI Insight 201

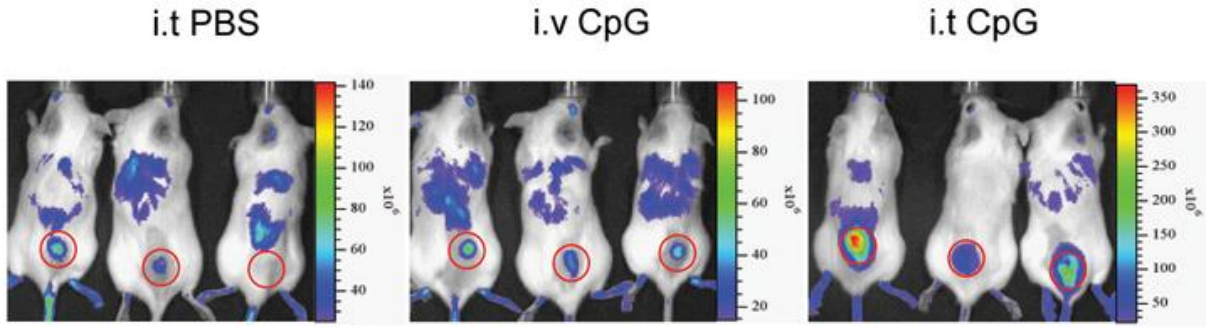
Intratumoral *vs.* Subcutaneous TLR7/8 Agonist: Route of Administration Is Critical



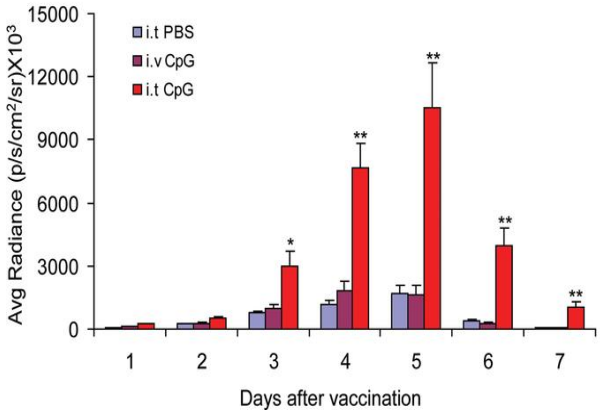
Mullins *et al.* JITC 2019

Intratumoral *vs.* Intravenous TLR9 Agonist: Route of Administration Is Critical

intratumoral Luciferase^{+} tumor-specific CD8+ T cells



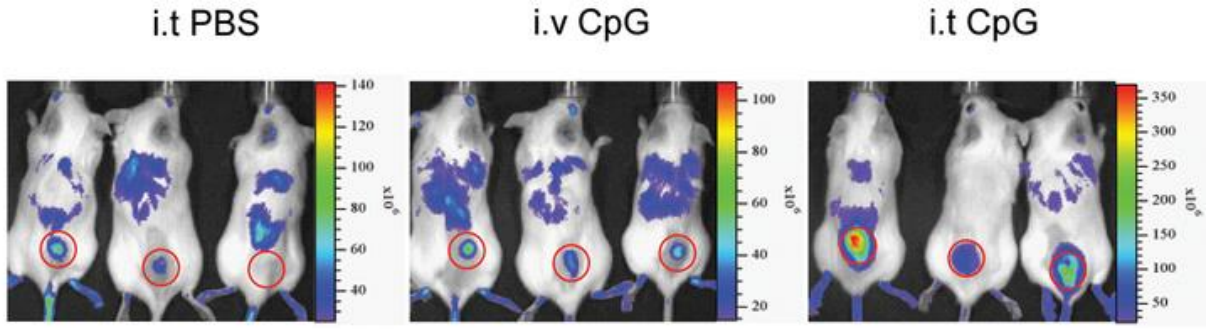
intratumoral T cells



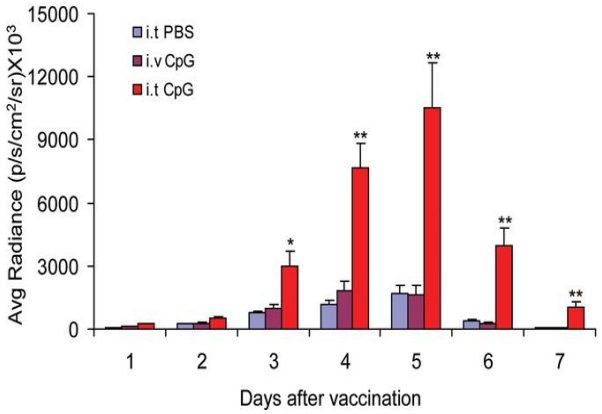
Lou et al., J Immunother. 2011

Intratumoral *vs.* Intravenous TLR9 Agonist: Route of Administration Is Critical

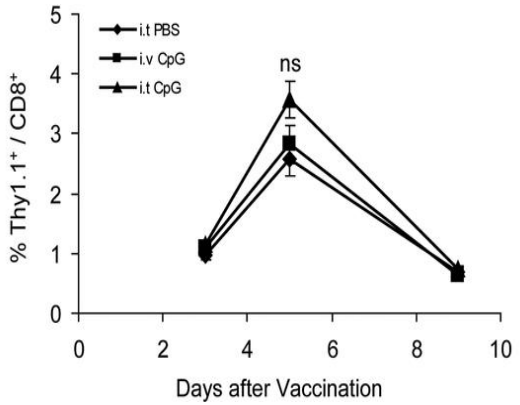
intratumoral Luciferase^{+} tumor-specific CD8+ T cells



intratumoral T cells



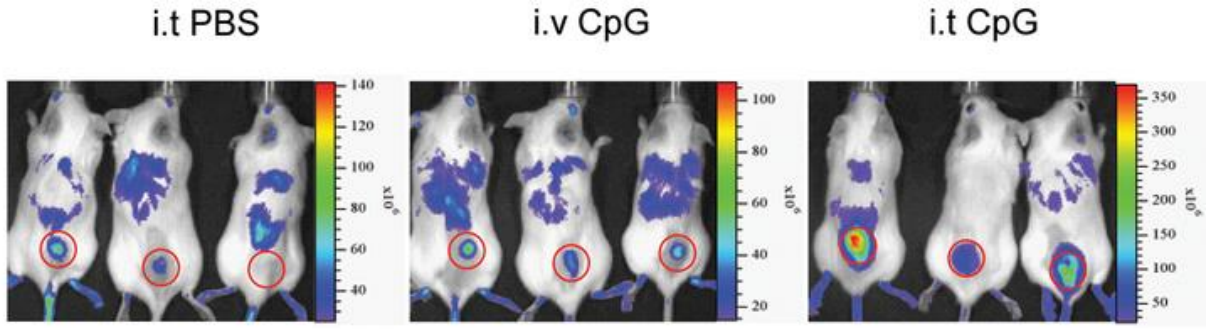
circulating T cells



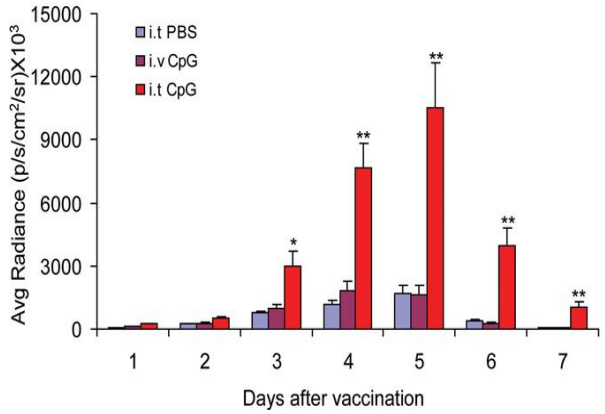
Lou et al., J Immunother. 2011

Intratumoral *vs.* Intravenous TLR9 Agonist: Route of Administration Is Critical

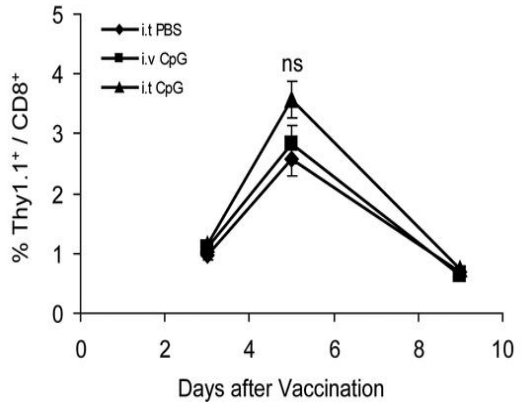
intratumoral Luciferase^{+} tumor-specific CD8+ T cells

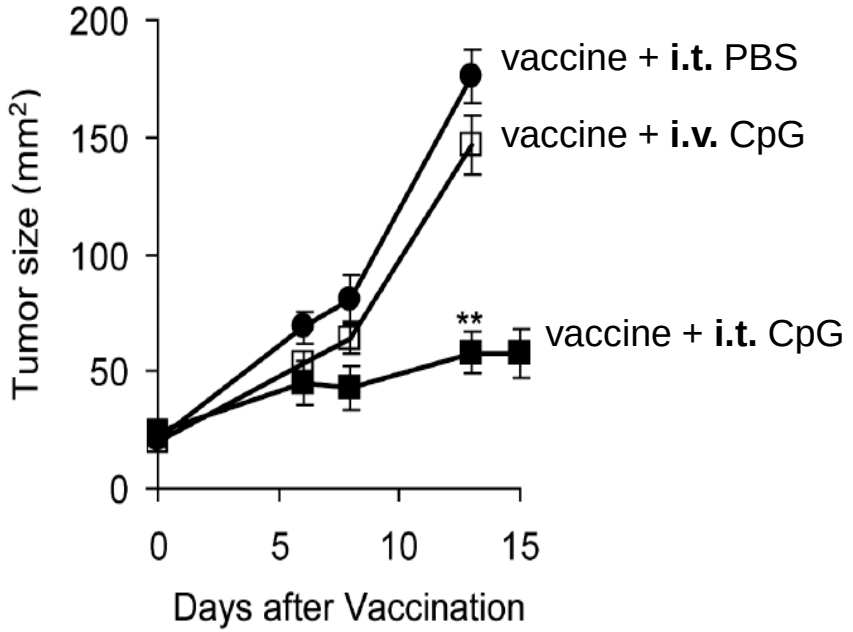


intratumoral T cells



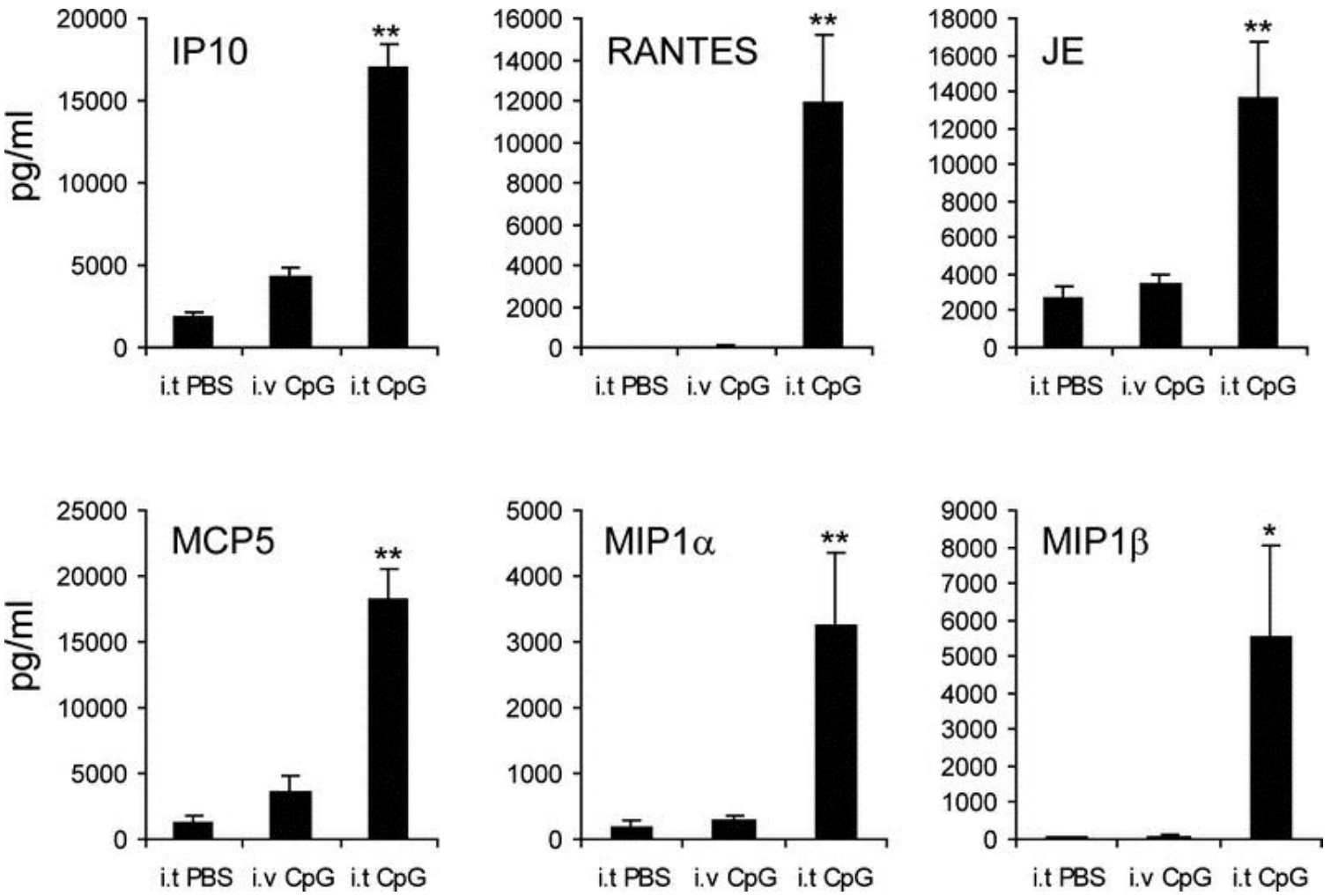
circulating T cells





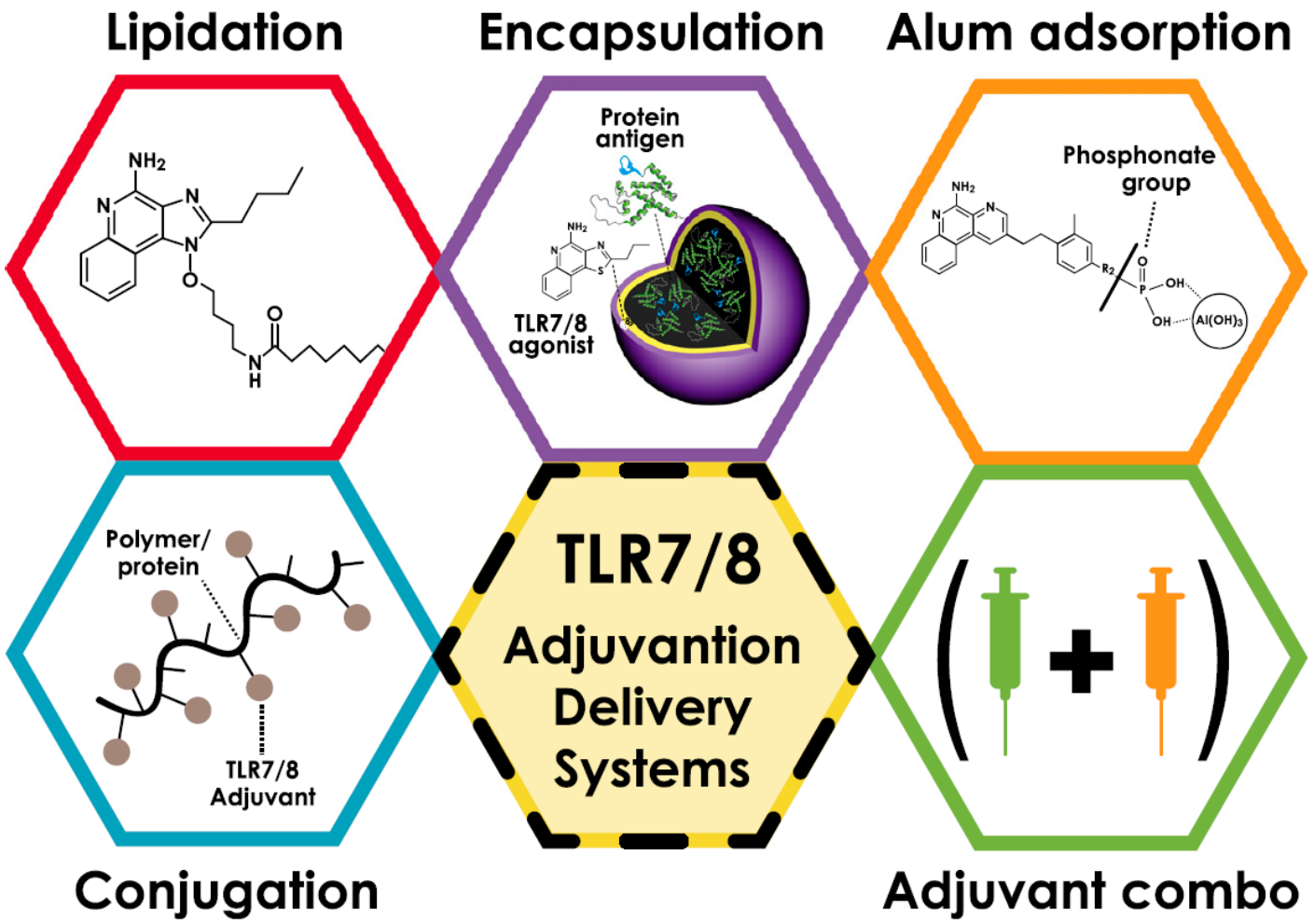
Lou et al., J Immunother. 2011

Intratumoral *vs.* Systemic TLR9 Agonist: Intratumoral Chemokines



Lou et al., J Immunother. 2011

Approaches to TLR 7/8 Agonist Delivery



Dowling et al., *Immunohorizons* 2018

Clinical Studies

Journal of Translational Medicine BioMed Central

Research

Open Access

Immune-mediated changes in actinic keratosis following topical treatment with imiquimod 5% cream

Abel Torres[1], Leslie Storey[1], Makala Anders[1], Richard L Miller[2], Barbara J Bulbulian[2], Jizhong Jin[2], Shalini Raghavan[2], James Lee[3], Herbert B Slade[3] and Woubalem Birmachu*[2]

Journal of Translational Medicine 2007,

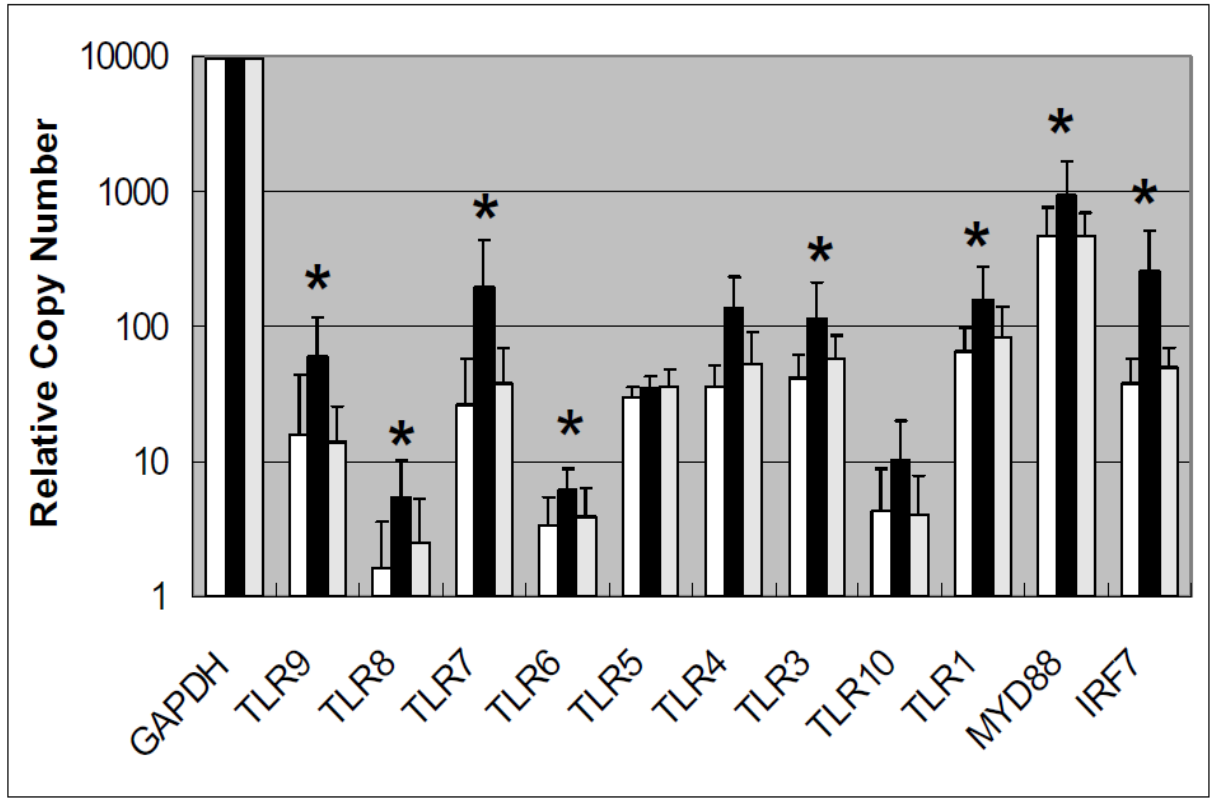
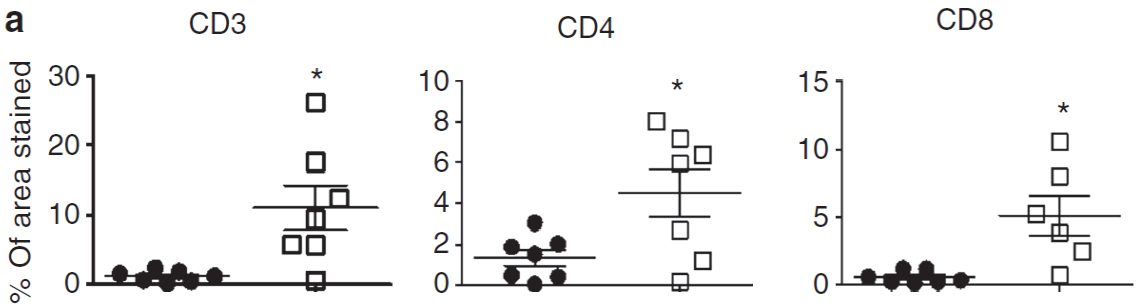


Figure 3
Basal *TLR*, *IRF7*, and *MyD88* gene expression in skin biopsies as determined by real time RT-PCR. White bars represent pre-treatment AK, black bars represent during imiquimod treatment (maximum response value from week 1, week 2 and week 4 treatment times), and hatched bars represent 4-weeks post treatment. Relative copy number was determined as outlined in Methods and Materials section. Asterisks indicate those genes that had p-values < 0.05 in the ANOVA, comparing expression in pretreatment AK samples to the maximum response expression in samples from subjects (n = 13) during imiquimod treatment. [See Additional file 2].

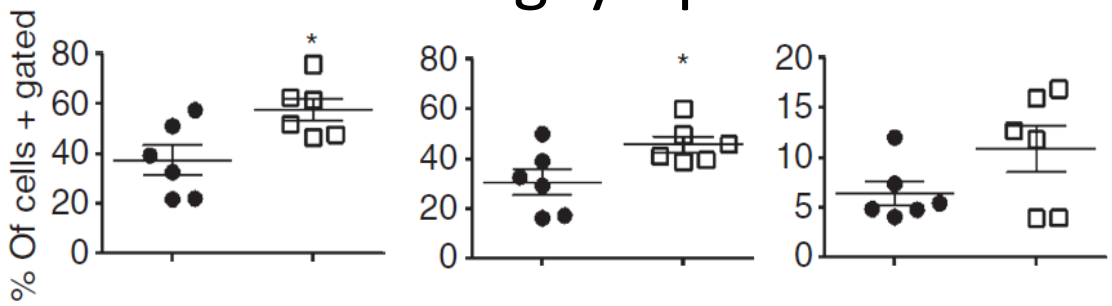
Imiquimod-induced Regression of Superficial Melanoma

Figure 1. Melanoma site of "responder", before and after treatment with imiquimod. Patient applied topical imiquimod to tumor site starting on day −14, and ending on day −1, before surgery on day 0.

tumor tissue



draining lymph node



Narayan *et al.*, Journal of Investigative Dermatology (2012) 1

Cancer Therapy: Clinical
See related commentary by Kohrt, p. 6571
Clin Cancer Res; 18(24) December 15, 2012

Topical TLR7 Agonist Imiquimod Can Induce Immune-Mediated Rejection of Skin Metastases in Patients with Breast Cancer

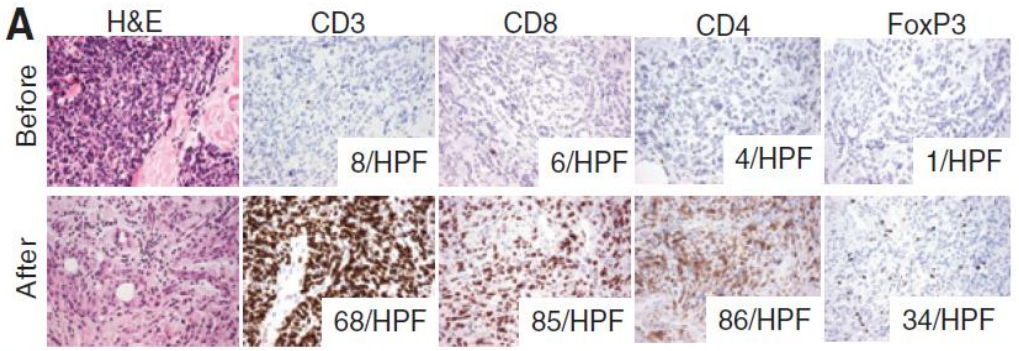
Sylvia Adams[1], Lina Kozhaya[2], Frank Martiniuk[1], Tze-Chiang Meng[7], Luis Chiriboga[3], Leonard Liebes[1], Tsivia Hochman[4], Nicholas Shuman[1], Deborah Axelrod[5], James Speyer[1], Yelena Novik[1], Amy Tiersten[1], Judith D. Goldberg[4], Silvia C. Formenti[6], Nina Bhardwaj[3], Derya Unutmaz[2], and Sandra Demaria[3]

Table 3. Local antitumor response

Response	$ROI^{change} = (ROI^{posttreatment}/ROI^{pretreatment}) \times 100\%$	Patients (%)
CCR	Absence of any detectable residual disease	None
PR	>0% to <50%	2 (20%)
SD	≥50% to <100%	5 (50%)
NR	≥100% to <125%	1 (10%)
PD	≥125% or new skin lesions	2 (20%)

NOTE: Percentage change in ROI after 8-week imiquimod treatment ($n = 10$).
Abbreviations: ROI, region of interest; CCR, complete clinical response; PR, partial response; SD, stable disease; NR, no response; PD, progressive disease.

responder biopsy after 8 weeks of imiquimod



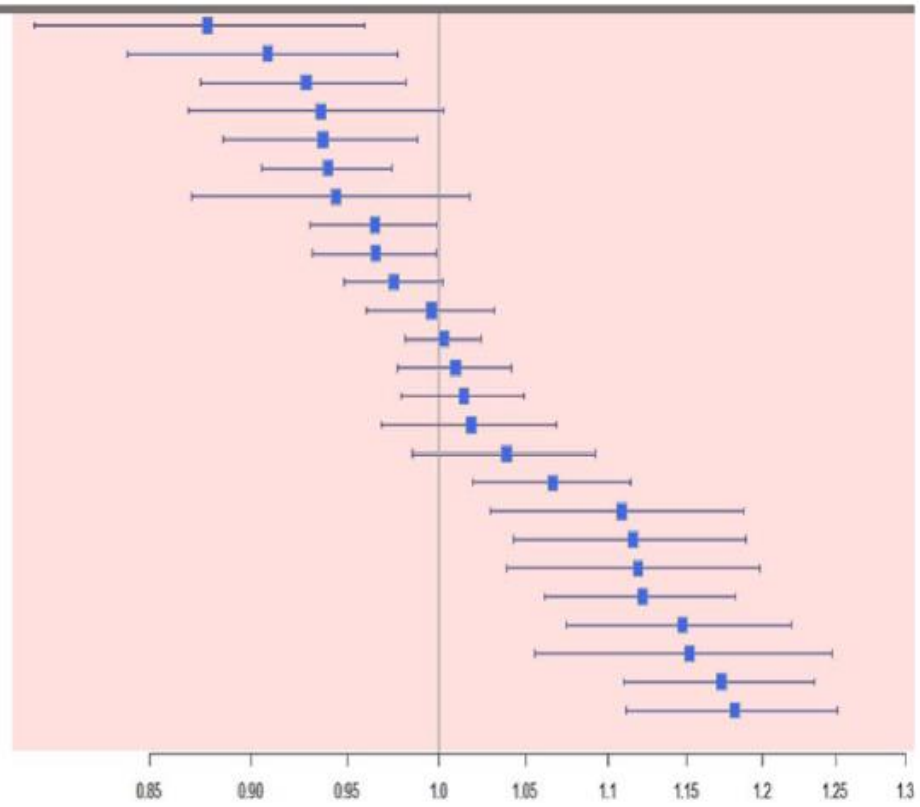
Imiquimod-induced Regression of Breast Cancer Skin Metastases

A.

Post- vs Pre-treatment
all samples

mRNA profiling

Leucocyte subpopulation	*p* value	Fold change (exp)
Th17 cell	0.11	0.88
Mast cell	0.163	0.91
NK CD56bright cell	0.167	0.93
iDC	0.328	0.94
NK cell	0.205	0.94
Tcm	0.0718	0.94
pDC	0.443	0.94
Th2 cell	0.306	0.96
TFH	0.298	0.97
T helper cell	0.362	0.98
Tem	0.921	1
Eosinophils	0.897	1
DC	0.78	1
Macrophages	0.695	1
Tgd	0.727	1
B-cell	0.483	1
NK CD56dim cell	0.172	1.1
Cytotoxic cell	0.192	1.1
Th1 cell	0.132	1.1
aDC	0.16	1.1
Neutrophils	0.0562	1.1
ICR_genes	0.058	1.1
Treg	0.142	1.2
T-cell	0.0111	1.2
CD8 T-cell	0.0171	1.2



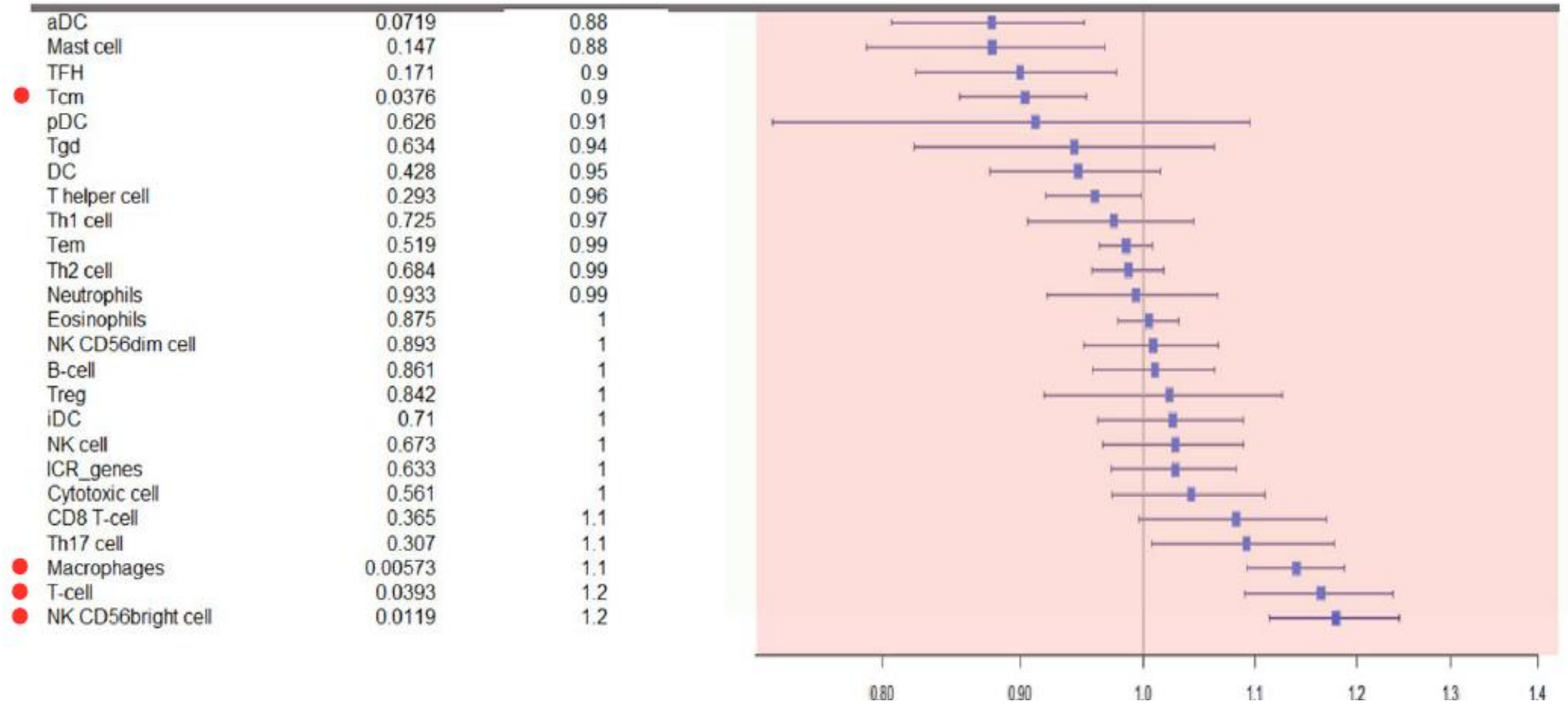
Rozenblit *et al.*, Scientific Reports, 2019

Imiquimod-induced Regression of Breast Cancer Skin Metastases

B.

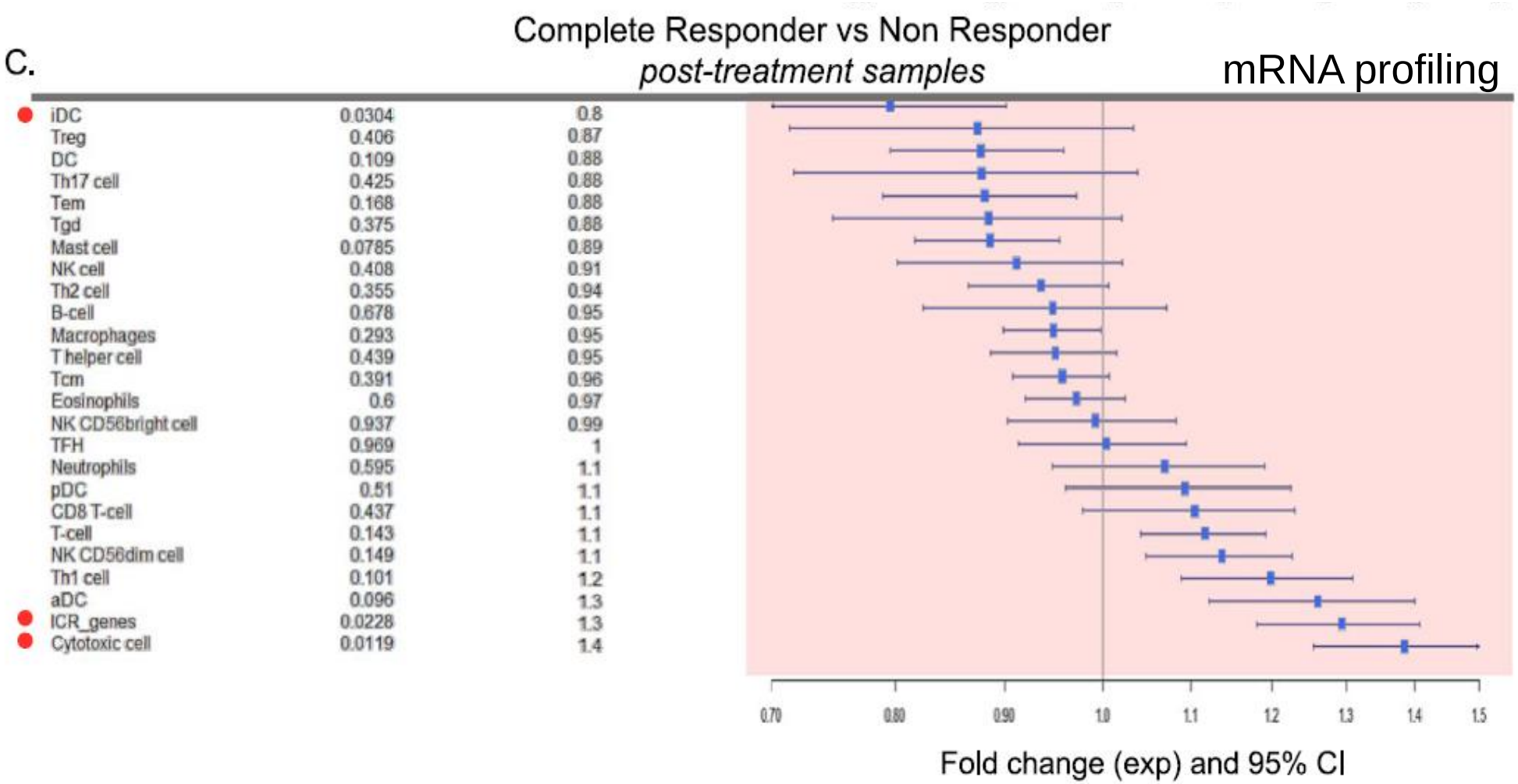
Complete Responder vs Non Responder
pre-treatment samples

mRNA profiling



Rozenblit *et al.,* Scientific Reports, 2019

Imiquimod-induced Regression of Superficial Melanoma



Rozenblit *et al.*, Scientific Reports, 2019

Some TLR7 & 8 Agonists In (Pre-)Clinical Development

Marketed	**Imiquimod/Aldara** (Medicis Pharmaceuticals) **TLR7 Agonist** Actinic Keratosis, Genital Warts, Basal Cell Carcinoma of Skin							
Registration	**Imiquimod/Aldara** (Medicis Pharmaceuticals) **TLR7 Agonist** Anal Cancer, Cervical Cancer, Vulvar Intraepithelial Neoplasia							
Phase II	**Imiquimod/Aldara** (Medicis Pharmaceuticals) **TLR7 Agonist** Liquid and Solid Tumors	**Resiquimod** (Spirig Pharma) **TLR7/8 Agonist** Anaplastic Astrocytoma, Anaplastic Astro-oligodendroglioma, Glioblastoma, Melanoma	**DPV 001** (UbiVac) **TLR 2,3,4,7,9 Agonist** Non Small Cell Lung Cancer	**VTX-2337/Motolimod** (Celgene, VentiRx) **TLR8 Agonist** Cancer	**CV 9202** (CureVac) **TLR7/8 Modulator** Non Small Cell Lung Cancer	**Vesimune** (UroGen Pharma) **TLR7 Agonist** Bladder Cancer		
Phase I	**Imiquimod/Aldara** (Medicis Pharmaceuticals) **TLR7 Agonist** Liquid and Solid Tumors	**CV 8102** (CureVac) **TLR7/8 Agonist** Adenoid-Cystic-Carcinoma, Head and Neck Cancer, Melanoma, Squamous Cell Carcinoma of Skin	**DPV 001** (UbiVac) **TLR 2,3,4,7,9 Agonist** Prostate Cancer	**DSP 0509** (Boston Biomedical; Sumitomo Dainippon Pharma) **TLR7 Agonist** Solid Tumors	**Telratolimod/MEDI9197/3M-052** (3M Drug Delivery Systems/MedImmune) **TLR7/8 agonist** Cutaneous T cell lymphoma, Solid Tumors	**BDB001** (Birdie Biopharmaceuticals) **TLR Agonist** Solid Tumors	**NKTR 262** (Nektar Therapeutics) **TLR7/8 Agonist** Bladder Cancer, CRC, RCC Melanoma, Merkel Cell Carcinoma, Ovarian Cancer, Soft Tissue Sarcoma, TNBC	**LHC165/NJH395** (Novartis) **TLR7 agonist** Solid Tumors
Preclinical	**1V270** (University of California, San Diego, La Jolla, USA; Graduate School of Tokyo Medical and Dental University, Japan) **TLR7 Agonist** Lung metastases, Breast cancer, melanoma, Pulmonary metastatic cancers	**DV 1001** (Dynavax Technologies) **TLR7/8 Agonist** Multiple malignancies	**3M-011** (Technische Universität Dresden, Germany) **TLR7/8 Agonist** Pancreatic cancer, Colon cancer	**DSR-6434** (University of Manchester, Manchester Academic Health Sciences Centre, United Kingdom) **TLR7 Agonist** Colon cancer, Renal cell carcinoma	**DSR-29133** (Sumitomo Dainippon Pharma, Osaka, Japan; AstraZeneca Pharmaceuticals Ltd.) **TLR7 Agonist** Colon cancer, Renal cell carcinoma, Osteosarcoma	**SC1** (BioNTech AG , 4SC Discovery GmbH) **TLR7 Agonist** Lymphoma, Renal cell carcinoma, Cancer	**SZU-101** (Shenzhen University, Shenzhen, China) **TLR7 Agonist** Breast carcinoma, Gastric cancer, T cell lymphoma	**SM-276001** (Dainippon Sumitomo Pharmaceuticals) **TLR7 Agonist** Tumor

Conclusions

- Intratumoral TLR7/8 agonists stimulate APCs to activate tumor-specific T cells
- Other immune cells are also activated/attracted, and contribute to tumor killing
- Selective intratumoral localization of TLR agonist is important
- Intratumoral TLR7/8 agonists combine well with other immunological and non-immunological therapeutics, including checkpoint blockade
- Multiple agonists of TLR7 and/or TLR8 are under clinical development

Thank You