CAR-T Clinical Trials: New Directions in Biomarkers

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Chief Medical Officer

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What is the Future of CAR-T?

- Initial CAR-T outcomes have been revolutionary (e.g., high 3-5yr disease-free survival rates)
  - But, the field is nascent and there is extraordinary remaining potential
  - And, products are complex- in addition to the differences in constructs and methods, each product is a living drug liable to intra-product variability in patient (e.g., source T-cells, host environment and tumor) & manufacturing
- Advances in genetic engineering and manufacturing techniques provide markedly greater potential for rationale improvement than in classical drug development
- What is being done to advance the field?
  - Novel binding domains and multi-CARs
  - Vectors- viral and non-viral (transposons)
  - Safety switches
  - Selection
  - Editing (KOs)
  - Biomarkers- inform all above
    - For CAR-T cells
    - For patients (host and apheresis)
    - For disease
    - For in vivo activity

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Biomarkers

- Predictive biomarkers are well established in oncology, particularly when a drug specifically targets an oncogenic driver
- CAR-T cells are a nascent and far more complex field
- Although CAR-T cells target tumor selective antigens, correlations between antigen levels and efficacy have been poor
- Each patient may not only have a unique tumor genotype / phenotype, but a unique drug (T cell) genotype / phenotype and host immunologic milieu
- Thus, benefit in assessing each tumor, CAR-T and host

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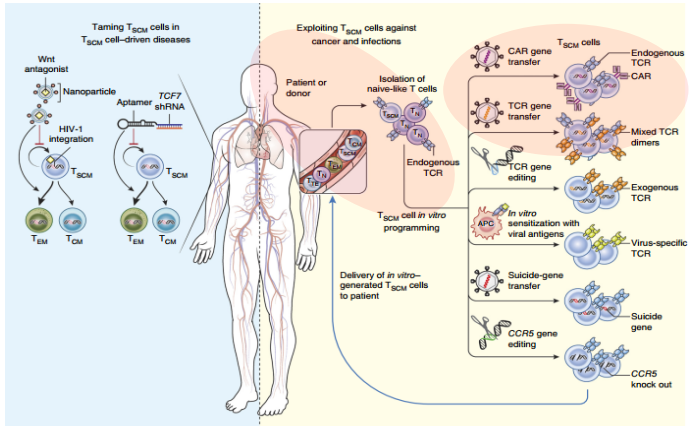
T stem-cell memory / central memory (Tscm/Tcm)

T_{SCM} May Be Key to Safe, Potent and Durable Responses

- "The extreme longevity, the robust proliferative potential and the capacity to reconstitute a wide-ranging diversity of the T cell compartment make **the T_{SCM} cell type an ideal cell population to employ in adoptive immunotherapy**"



T memory stem cells in health and disease

Luca Gattinoni[1], Daniel E Speiser[2], Mathias Lichterfeld[3] & Chiara Bonini[4,5]
18 VOLUME 23 | NUMBER 1 | JANUARY 2017 NATURE MEDICINE

- **Correlates with CAR-T clinical response**
  - *Melenhorst J. et al., UPenn (2017) 20th ASGCT*
  - *Basu et al., Adaptimmune (2017) CAR-TCR Summit*
  - *T_{CM}: Larson, Juno (2018) AACR*
  - *Bot A., et al., Kite (2018) SITC*
  - *T_{SCM} TIL: Beatty M., Moffitt (2018) SITC*
  - *T_{CM}: Fraietta J. et al., UPenn (2018) TET2 Disruption, PMID: 29849141*

	T_N	T_{SCM}	T_{CM}	T_{EM}	T_{TE}
CD45RA	+	+	-	-	+
CD45RO	-	-	+	+	-
CCR7	+	+	+	-	-
CD62L	+	+	+	-	-
CD28	+	+	+	+/-	-
CD27	+	+	+	+/-	-
IL-7Rα	+	+	+	+/-	-
CXCR3	-	+	+	-	-
CD95	-	+	+	+	+
CD11a	-	+	+	+	+
IL-2Rβ	-	+	+	+	+
CD58	-	+	+	+	+
CD57	-	-	-	+/-	+

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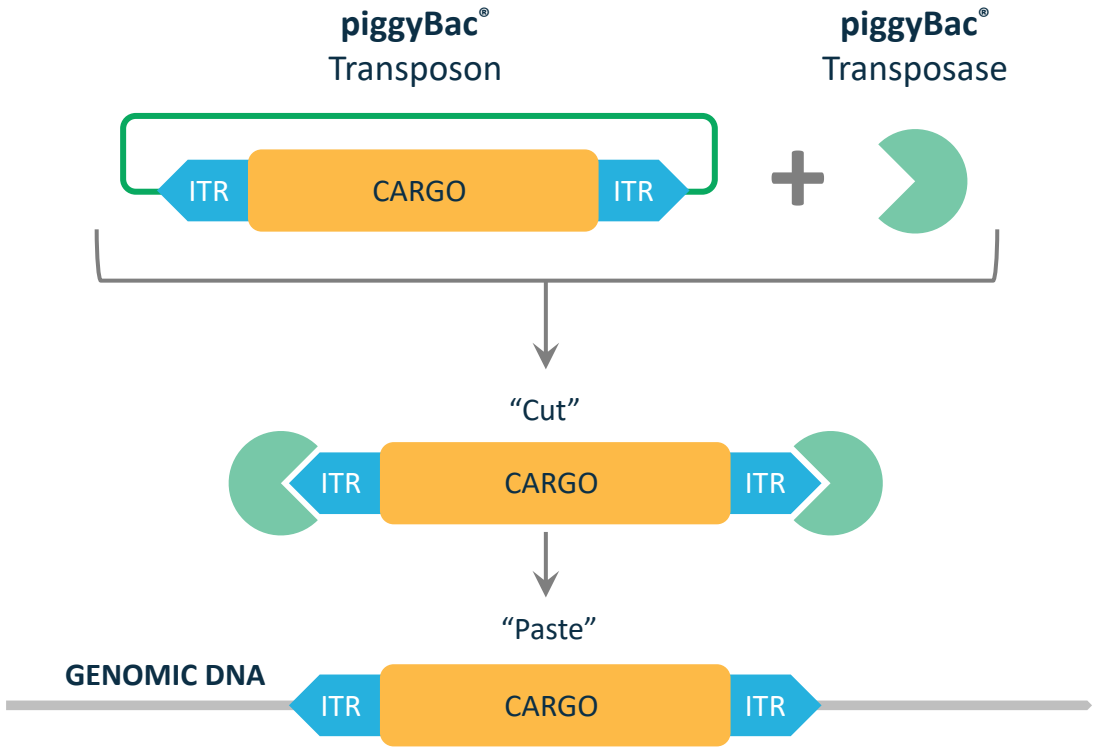
PiggyBac® DNA Transposon System

piggyBac® is a Superior DNA Delivery System for Developing CAR-T and Other Gene Therapy products

- **Unprecedented cargo capacity (>30X lentivirus)** – three-in-one transgene and possibility of multiple CAR or TCR molecules
- Non-viral delivery system **– non-oncogenic and non-mutagenic**
- **High insertion efficiency** and **stable transgene expression**
- **Faster** to clinic with **lower cost** than viral methods
- **Substantial IP portfolio** with no dominant or competing IP
- Creates products with highly desirable **T Stem Cell Memory (Tscm) Phenotype**



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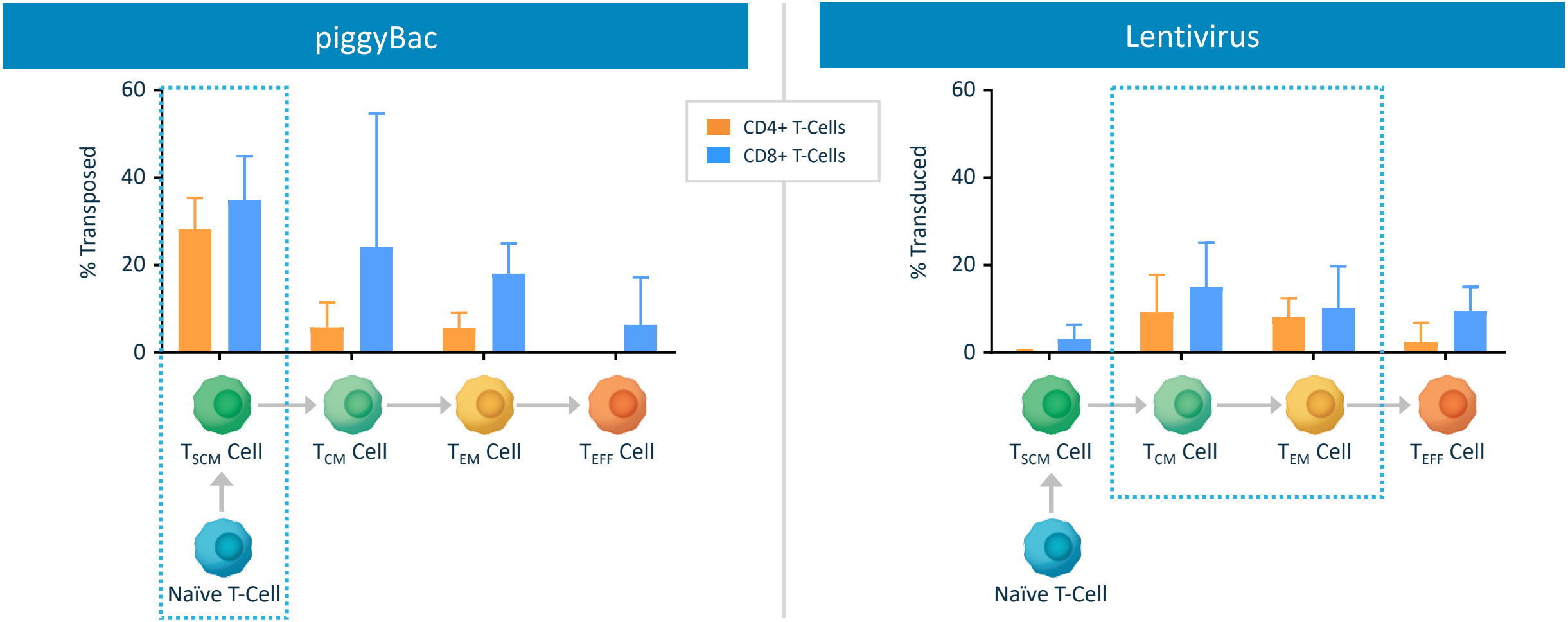
piggyBac® Preferentially Transposed *Early* T_{SCM} Cells; Lentivirus Transduced *More Differentiated* T-Cells In Preclinical Studies



We purified donor cells to these T-cell subsets and then performed optimized piggyBac or optimized lentivirus manufacturing on each subset

Percentage transposed (% GFP+) data are displayed for CD4+ T cells (CD3+CD4+CD8-) or CD8+ T cells (CD3+CD4-CD8+) within the final cell product

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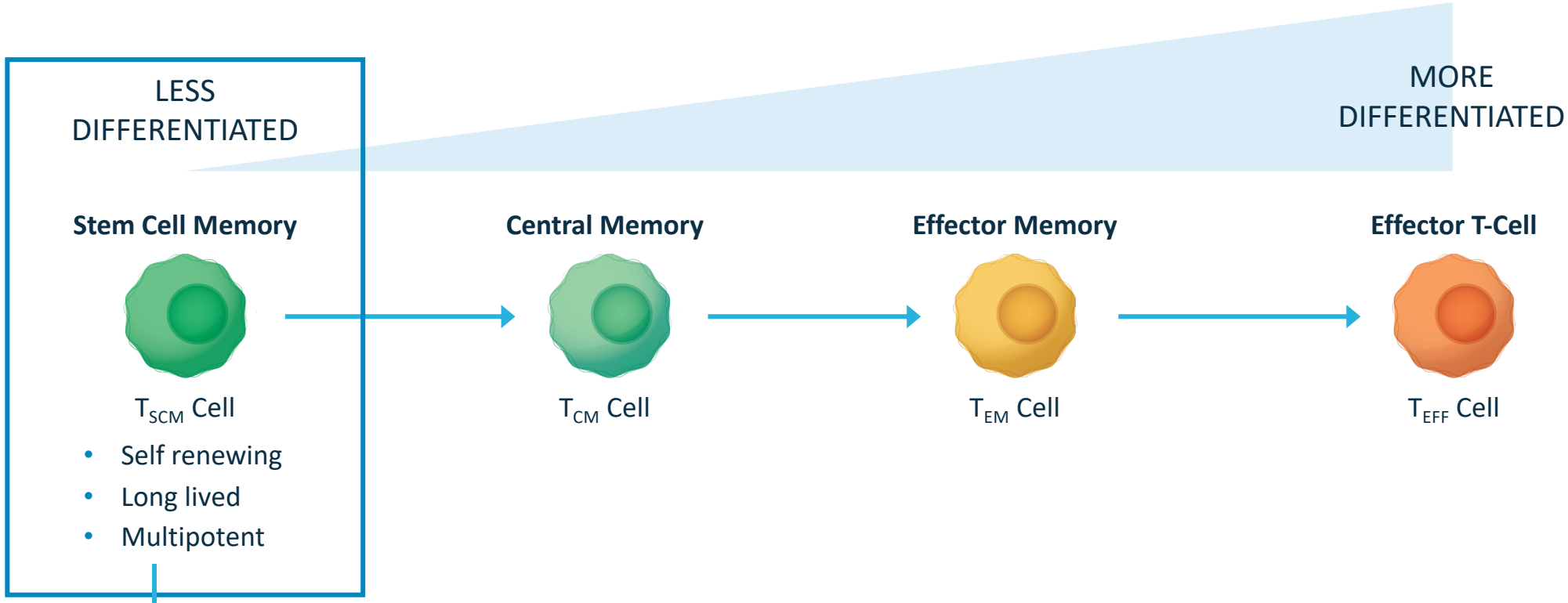
Not All T-Cells are Equal: The Importance of Stem Cell Memory Cells



THE POTENTIAL BENEFITS

Products with High % of T_{SCM} Cells:

- More gradual tumor killing with less toxicity
- Better duration of response and potential for re-response
- Key to CAR-T success in solid tumors

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P-BCMA-101/P-PSMA-101: Three-In-One Transgene CAR-T products

1 CAR-T MOLECULE

Superior binding molecule

- Centyrin binder with **high-specificity binding to target**
- Fully human and **not susceptible to tonic signaling**

2 POSITIVE SELECTION

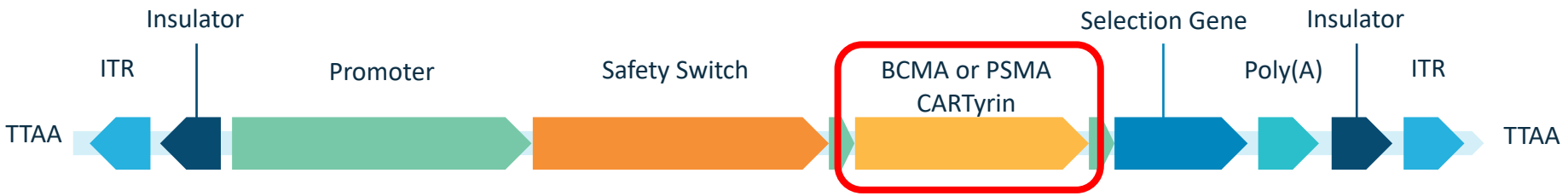
Drug resistance gene permits positive selection

- **~100% of T-cells** in final product express the CAR molecule
- Predicted to result in **better therapeutic index**

3 SAFETY SWITCH

Incorporates proprietary safety switch

- Rapid, dose-dependent elimination of engineered T-cells if needed
- Management of Cytokine Release Syndrome (CRS) or other AEs



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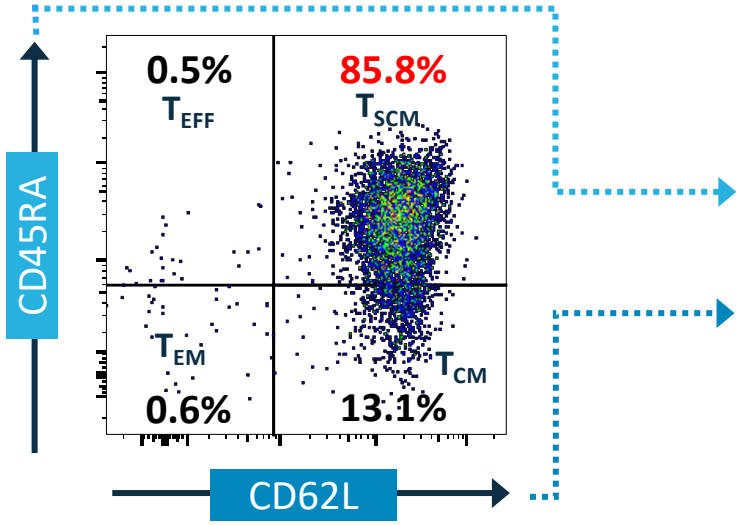
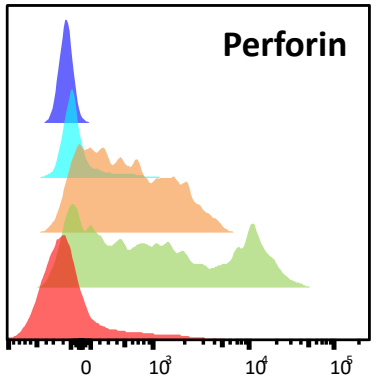
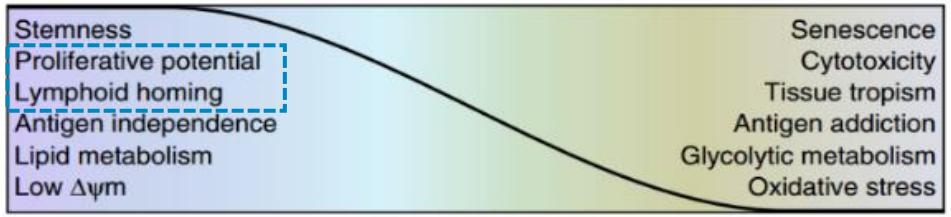
Stem Cell Memory T_{SCM} Phenotype in Poseida's Product Candidates



	T_N	T_{SCM}	T_{CM}	T_{EM}	T_{TE}	P-BCMA-101
CD45RA	+	+	-	-	+	+
CD45RO	-	-	+	+	-	-
CCR7	+	+	+	-	-	+
CD62L	+	+	+	-	-	+
CD28	+	+	+	+/-	-	+
CD27	+	+	+	+/-	-	+
IL-7Rα	+	+	+	+/-	-	+
CXCR3	-	+	+	-	-	+
CD95	-	+	+	+	+	+
CD11a	-	+	+	+	+	+
IL-2Rβ	-	+	+	+	+	+
CD58	-	+	+	+	+	+
CD57	-	-	-	+/-	+	-

Naïve/Tscm
Tcm
Tem
Teff
P-BCMA-101

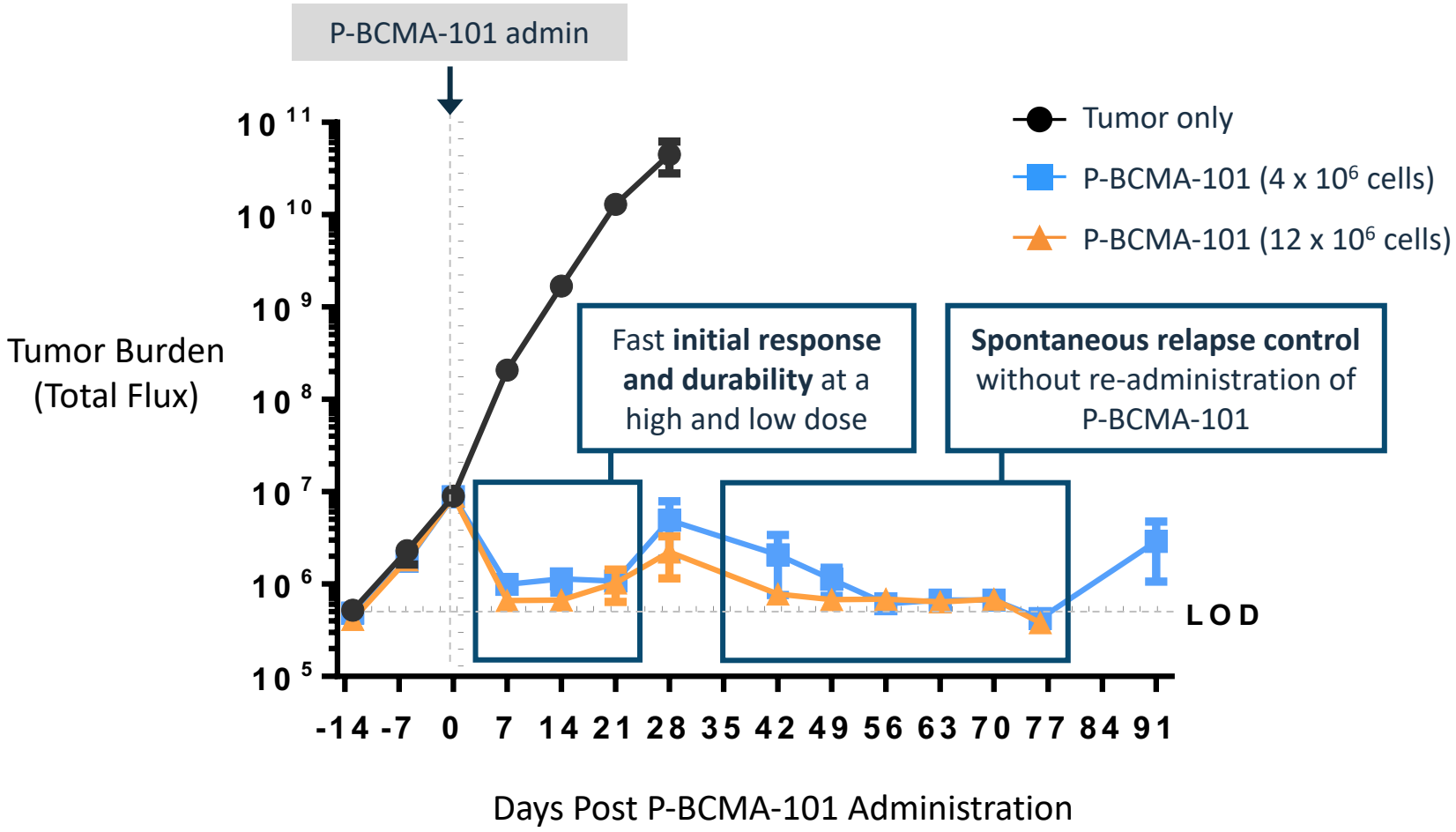




Adapted from Gattinoni et al. (2017) Nat. Med.

Our product more closely matches a T_{scm} phenotype when we do extensive cell surface markers and even intracellular markers

P-BCMA-101 Eliminated Tumors in Aggressive MM Cancer Model



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High % of T_{SCM} Cells: Unlocking Potential of CAR-T to Successfully Treat Solid Tumor Indications

Conventional Experience and Perception

- Poor CAR-T responses in solid tumors to date
- Rare instances with complete response (GBM, HCC) have occurred only after multiple administrations
- CAR-T can cause complete responses in solid tumors, but **numerous waves of more differentiated cells are required**

Our Approach

Our product candidates are comprised of a high percentage of **T_{SCM} cells**, which we believe hold the potential to engraft, self renew and **create wave after wave of more differentiated effector cells with one administration**

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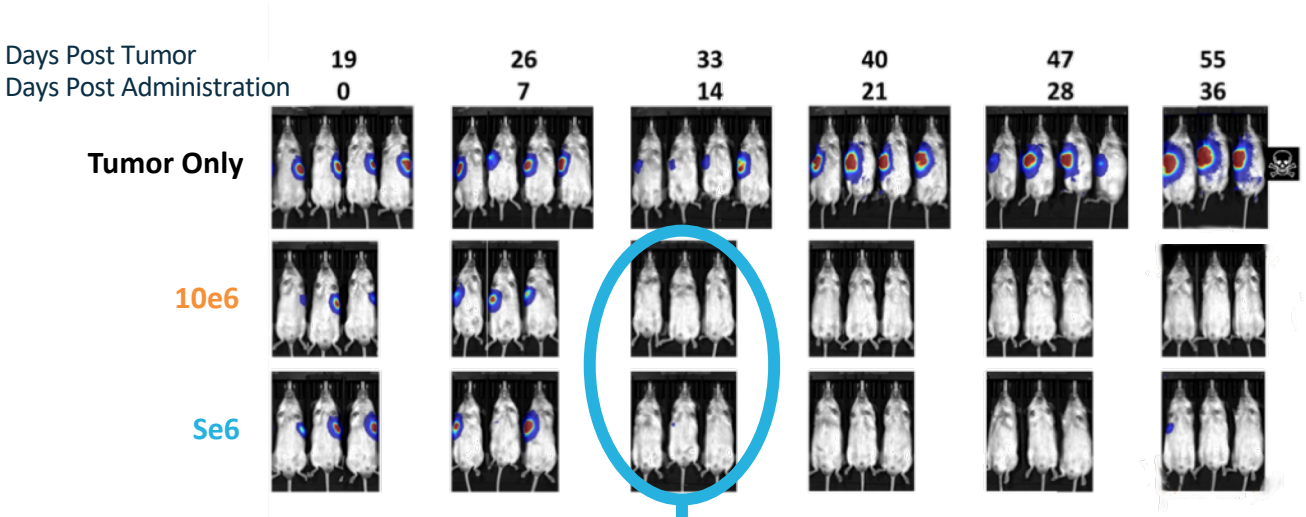
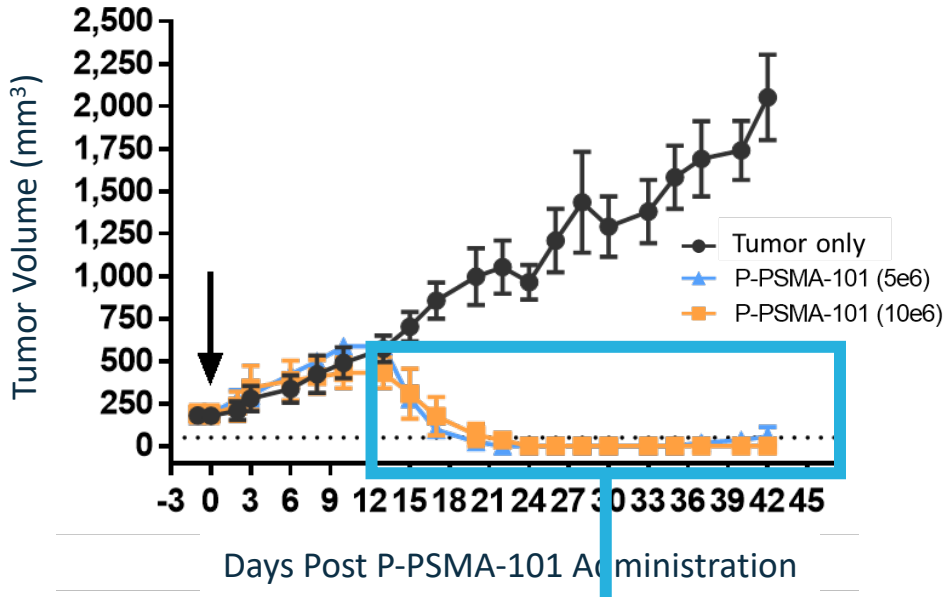
P-PSMA-101 Observed Potent In Vivo Activity

EFFICACY OF P-PSMA-101 IN PROSTATE CANCER MODEL (LNCaP.luc)

Imaging



Caliper Measurement



Tumor Elimination in 100% of Animals at Standard and Low Doses After ~ 2 Weeks

Data presented at SITC 2017. One animal in the low dose cohort relapsed later in the study.

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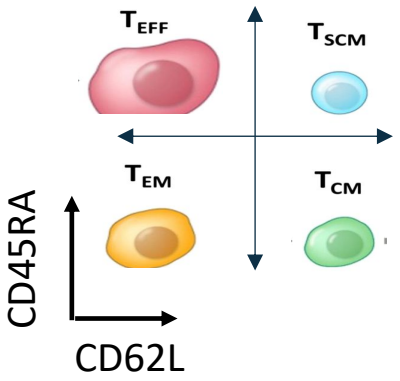
A Population of P-PSMA-101 T_{SCM} Persists

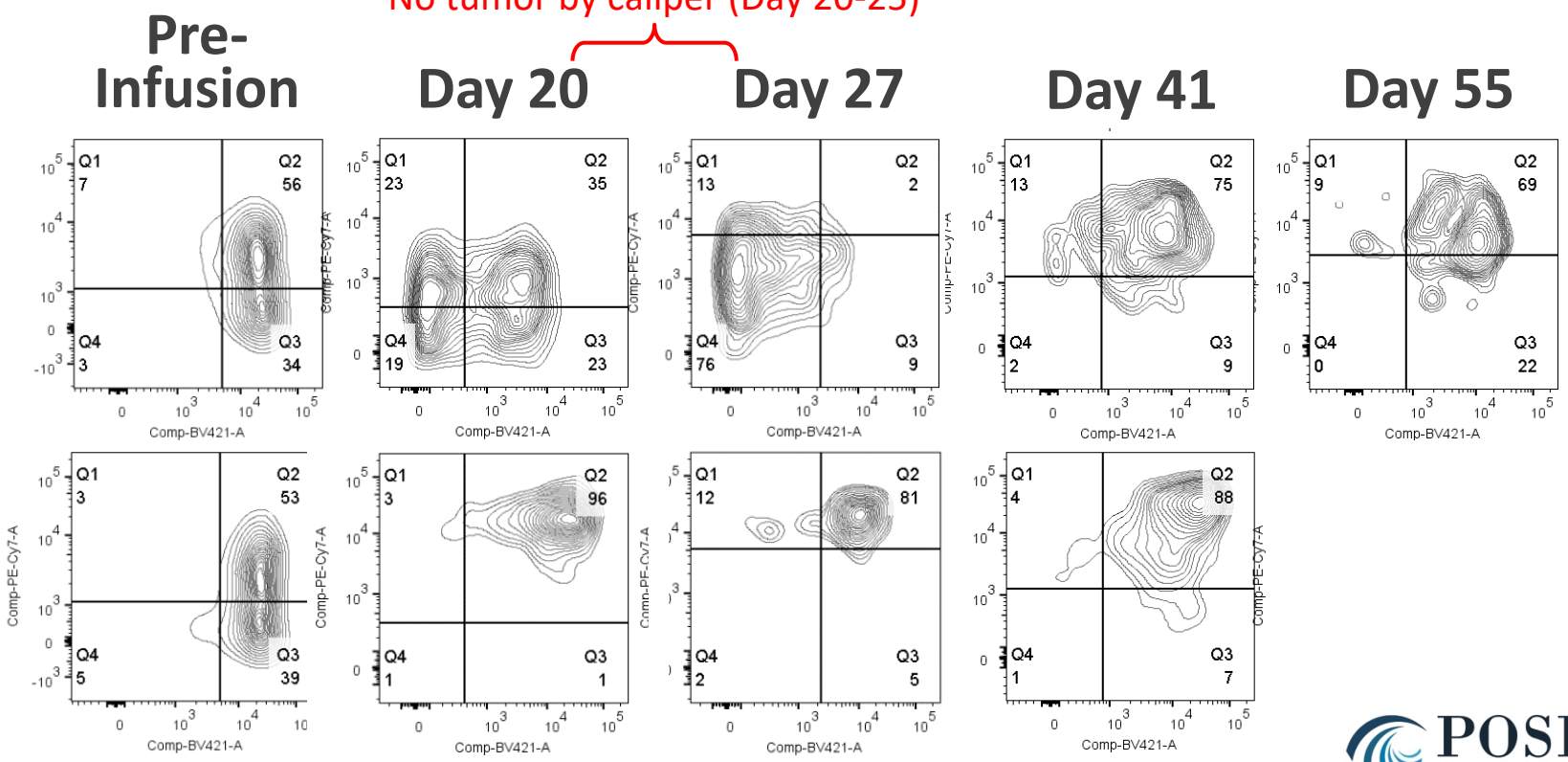
P-PSMA-101: Solid tumor (LNCaP) SC implantation in NSG mice

- P-PSMA-101 (T_{SCM}/T_{CM}) give rise to CARTyrin+ T_{CM}, T_{EM}, and Teff to attack solid tumor
- After solid tumor elimination, a population of P-PSMA-101 T_{SCM} persists



(T cells from blood)

No tumor by caliper (Day 20-25)



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Phase 1/2 Relapsed/Refractory Multiple Myeloma Clinical Trial (PRIME)

Phase 1 Trial Design

- Open Label, 3+3 Design, Single Ascending Dose Study
- 30 mg/m2 fludarabine + 300 mg/m2 cyclophosphamide x 3d lymphodepletion regimen
- P-BCMA-101 administered intravenously
  - Allowance for **multiple doses** and retreatment **after other CAR-Ts**
  - **Outpatient** administration allowed
- Up to 80 subjects

Phase 2 Trial Design

- Same schema as Phase 1
- P-BCMA-101 administered intravenously at 6-15 x 10^6 cells/kg
- 100 subjects

Clinical Trial Sites

Colorado Blood Cancer Institute- Tara Gregory, M.D.
Hackensack University Medical Center- David Siegel, M.D.
Johns Hopkins- Syed Abbas Ali, M.D.
Karmanos Cancer Institute- Abhinav Deol, M.D.
MD Anderson Cancer Center- Krina Patel, M.D.
Swedish Cancer Institute- William Bensinger, M.D.
Tennessee Oncology- Jesus G. Berdeja, M.D.
UC San Diego Moores Cancer Center- Caitlin Costello, M.D.
UC San Francisco- Nina Shah, M.D.
University of Chicago- Andrzej Jakubowiak, M.D.
University of Kansas Cancer Center- Siddhartha Ganguly, M.D.
University of Maryland- Aaron Rapoport, M.D.
University of Pennsylvania- Adam Cohen, M.D.

CIRM

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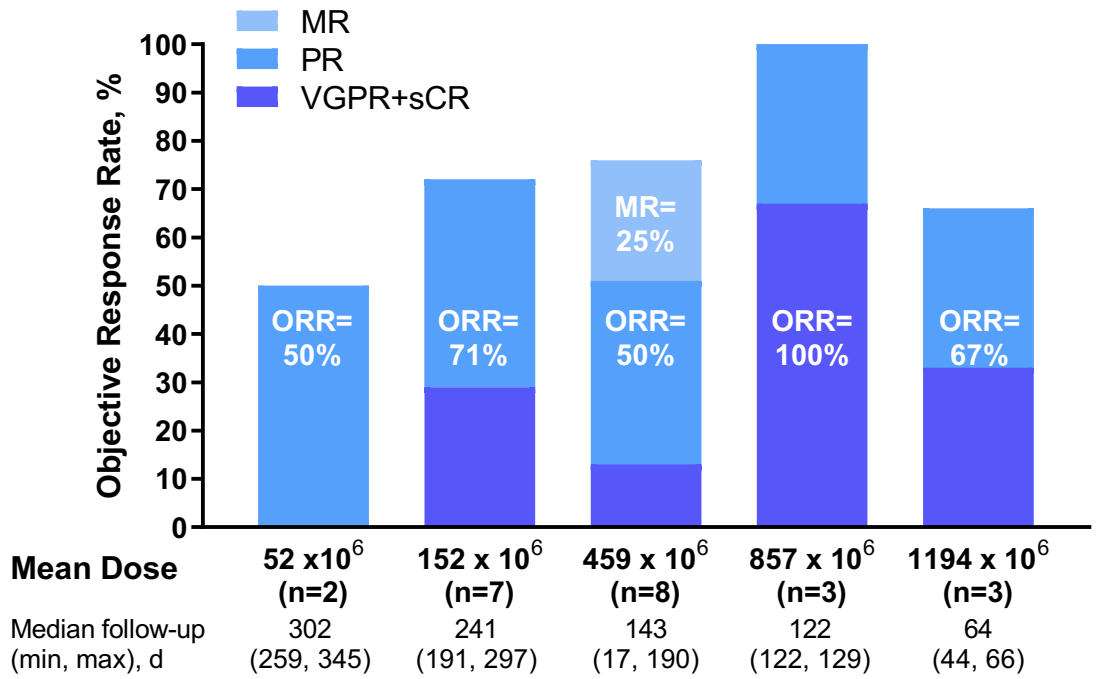
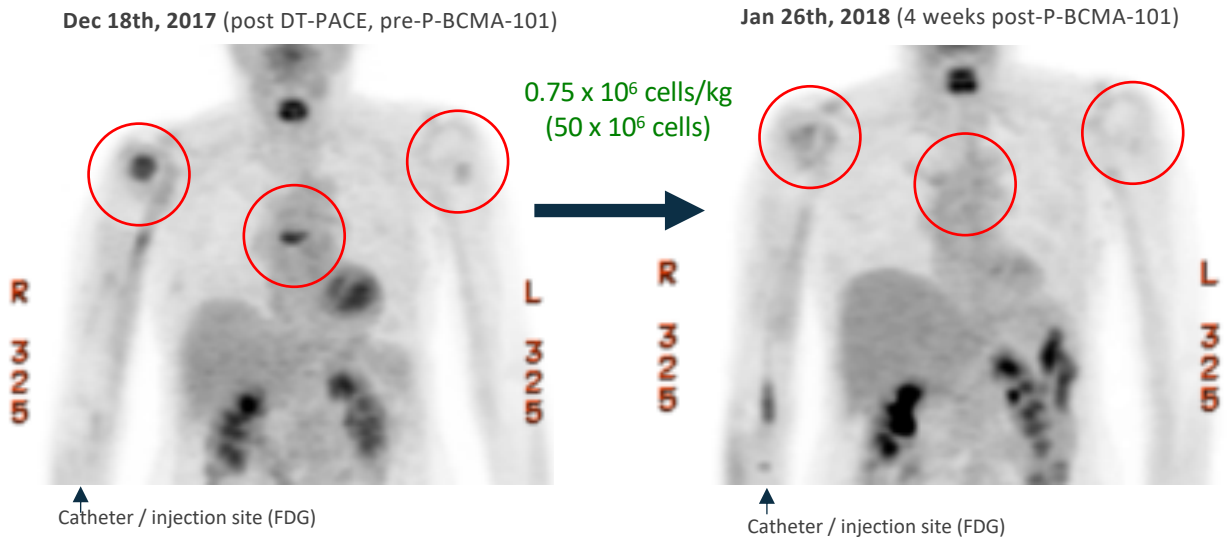
High Response Rates

Tumor Response in Evaluable Patients by Dose



Patient 105-002 PET



Oligosecretory disease, M-protein, SPEP, UPEP, FLC not measurable/within normal limits.

Data cutoff: January 31st, 2019. ORR, objective response rate, attaining sCR (inc. MRD-), CR, VGPR or PR, including confirmed and unconfirmed responses. Evaluable patients: evaluable first response assessment by IMWG m-protein criteria or PD/death.

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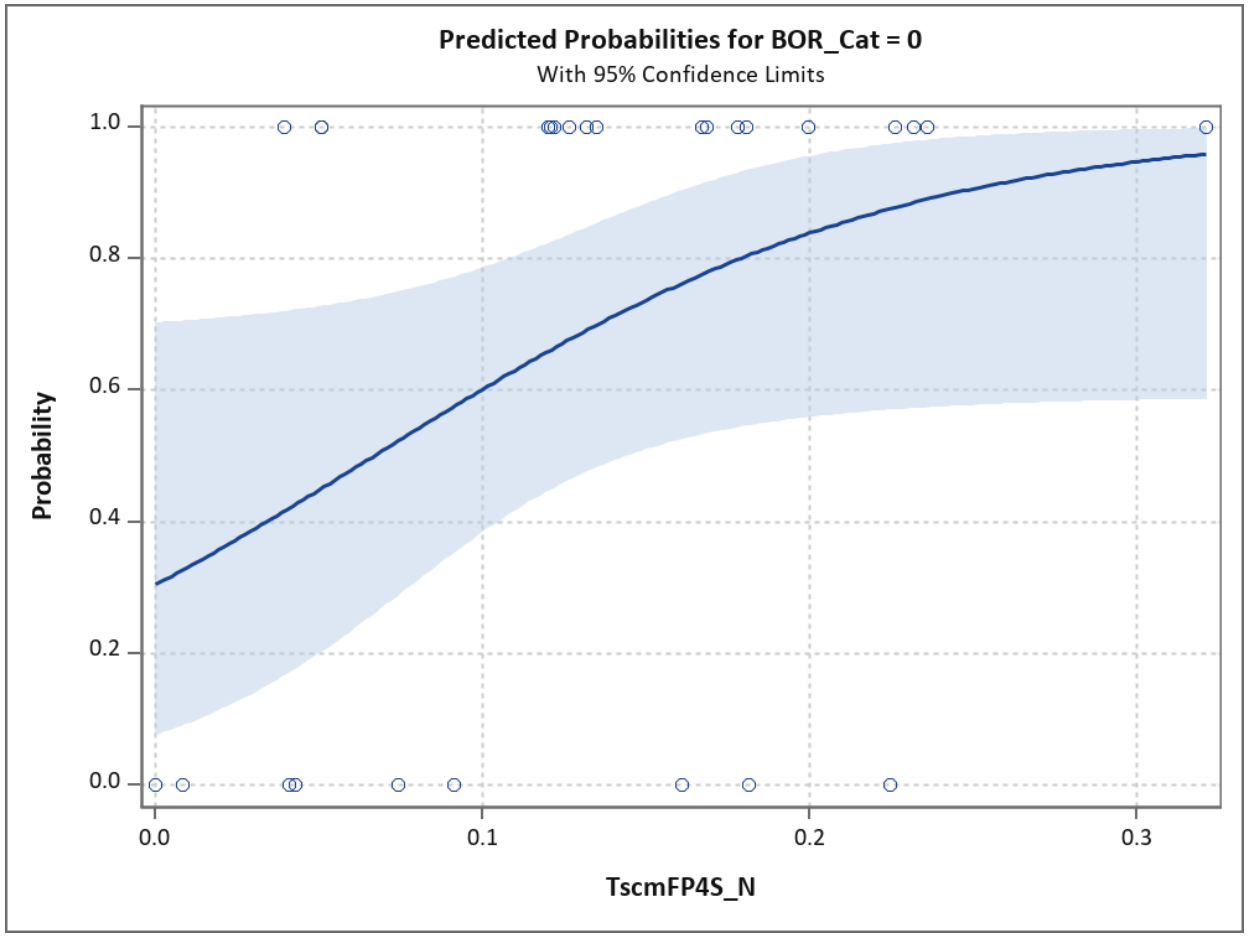
%Tscm Correlates with Response in Patients Treated with P-BCMA-101



Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq P-value
Intercept	1	-0.8267	0.8603	0.9233	0.3366
TscmFP4S	1	12.3798	6.5225	3.6025	**0.0577**

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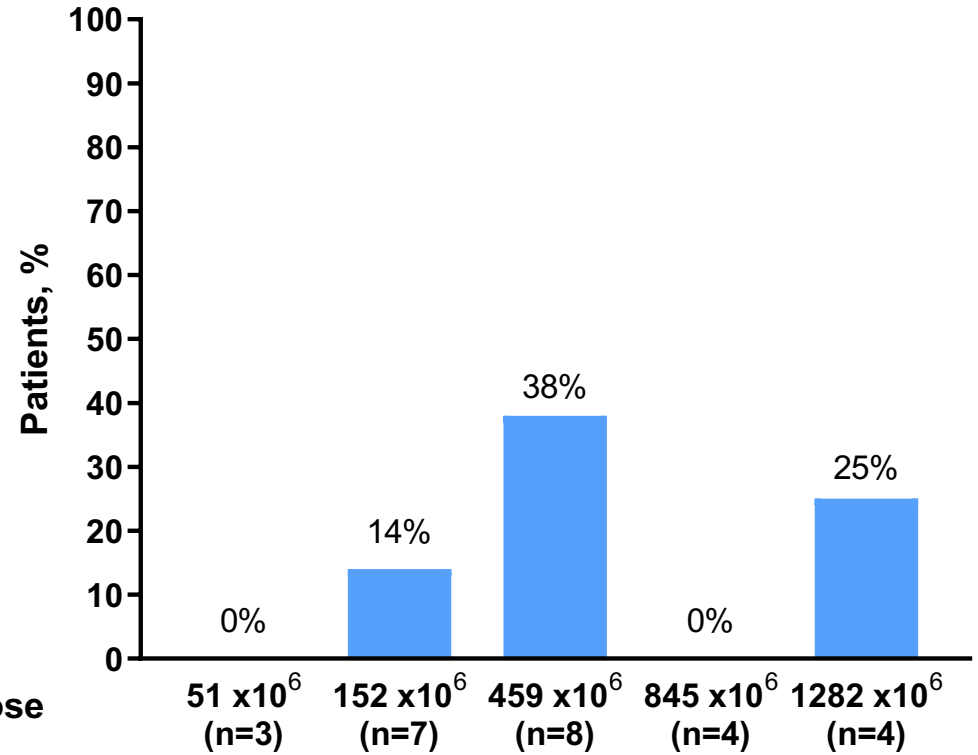
Adverse Events of Interest

Treatment-Emergent Adverse Events (n=26)

TEAE, n (%)	Overall	≥ Grade 3
Dose Limiting Toxicity (DLT)[a]	0	0
Cytokine Release Syndrome[a]	5 (19.2%)	0
Neurotoxicity[a]		
Grade 2 CRES with Grade 3 confusion (1 pt)	1 (3.8%)	1 (3.8%)
Neutropenia/Neutrophil count decreased[b]	17 (65.4%)	16 (61.5%)
Thrombocytopenia/Platelet count decreased[b]	11 (42.3%)	8 (30.8%)
Anemia	11 (42.3%)	9 (34.6%)
Infection[c]		
Overall	9 (34.6%)	4 (15.4%)
First month	6 (23.1%)	2 (7.7%)

Cytokine Release Syndrome By Dose Level



Data cutoff: January 31, 2019

[a]by investigator assessment

CRES based on confusion reported in a patient with baseline mental status decrement, tabulated in CRS & Neurotoxicity not including orthostatic dizziness or peripheral neuropathy/tremor

[b]subject counted once for either term

[c]includes events in the SOC Infections and Infestations. Subject counted once for any PT within the SOC.

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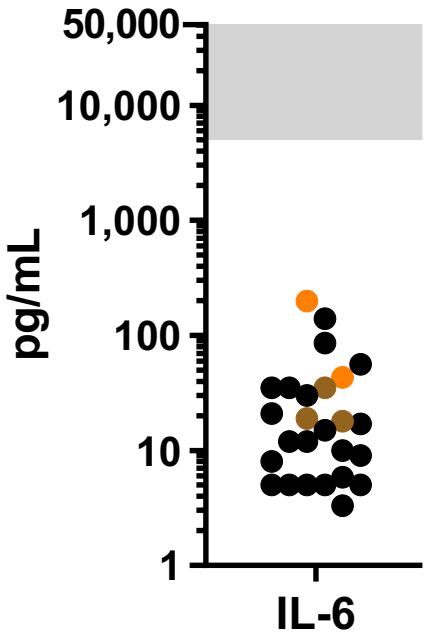
Cytokine Release Syndrome Minimal, IL-6 Low but Correlates

Cytokine Release Syndrome Parameters

Parameter	Dosed Patients (n=26)
Patients with a CRS event, n	5 (19.2%)
Maximum CRS grade	
None	21 (80.8%)
1	3 (11.5%)
2	2 (7.7%)
Median time to onset, d	8
Median duration, d	4

Peak IL-6 Levels After P-BCMA-101



Levels generally reported for patients with severe CRS[1]

- Grade 2 CRS assessed
- Grade 1 CRS assessed
- No CRS assessed

[1]*Maude et al., 2014*
Ali et al., 2016

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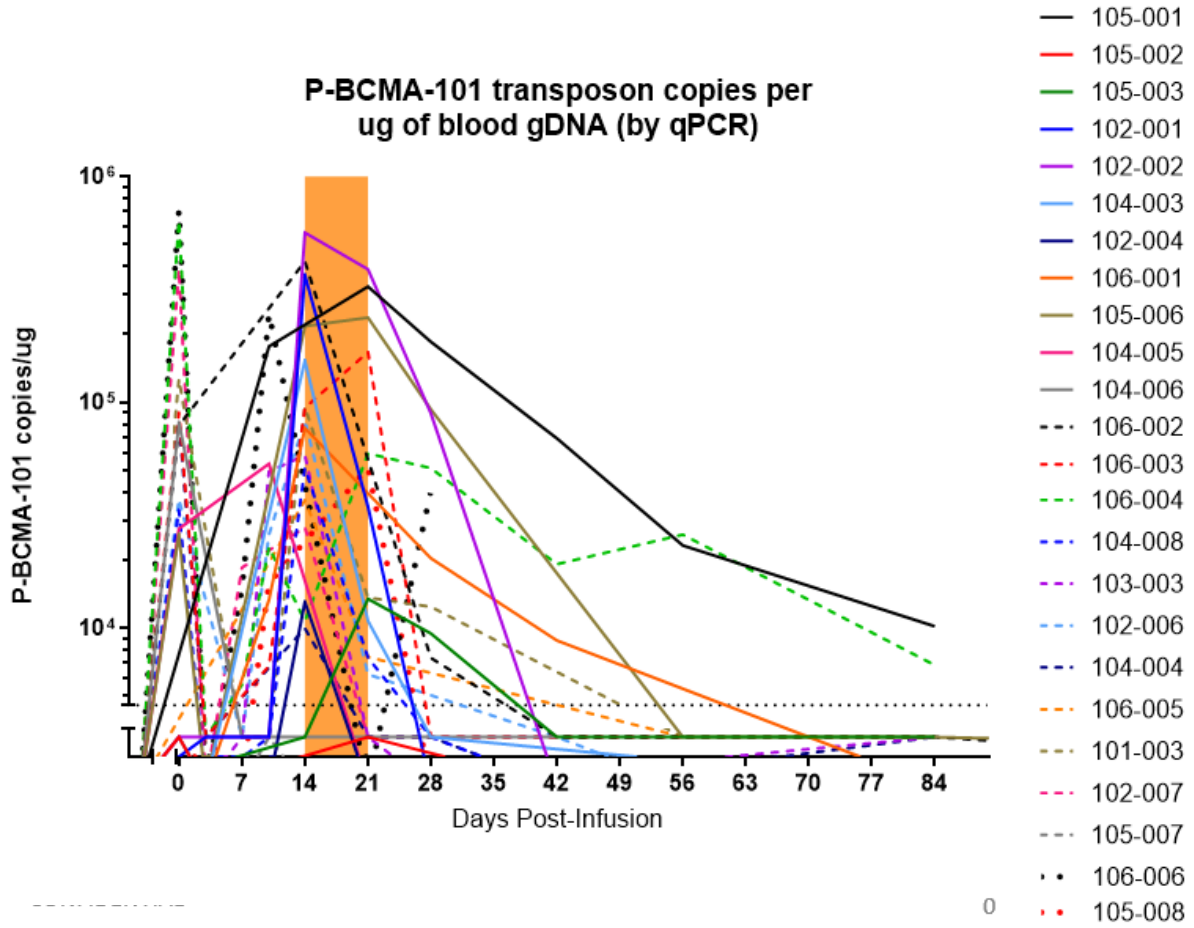
P-BCMA-101 CAR-T Cells in PB: Gradual Expansion

P-BCMA-101 in Peripheral Blood using PCR



- Many CAR-T products show **peak expansion between 5-14 days**
  - **Peak expansion of CAR-Ts often associated with CRS**
- P-BCMA-101 shows **peak expansion between 14-21 days**
  - P-BCMA-101 reaches peak expansion gradually **without CRS**

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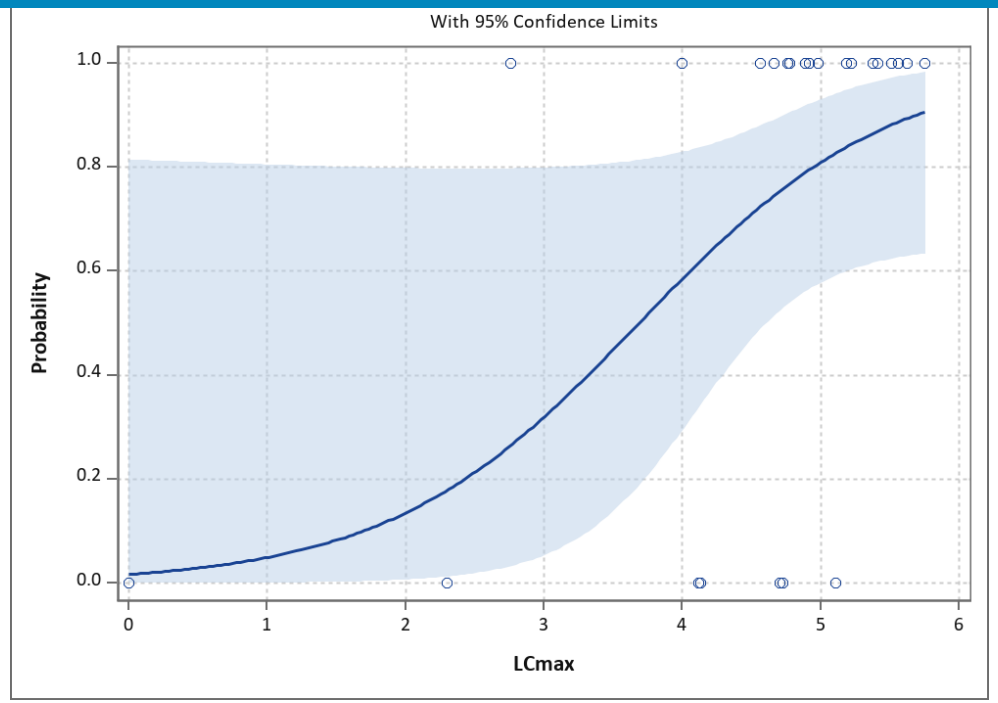
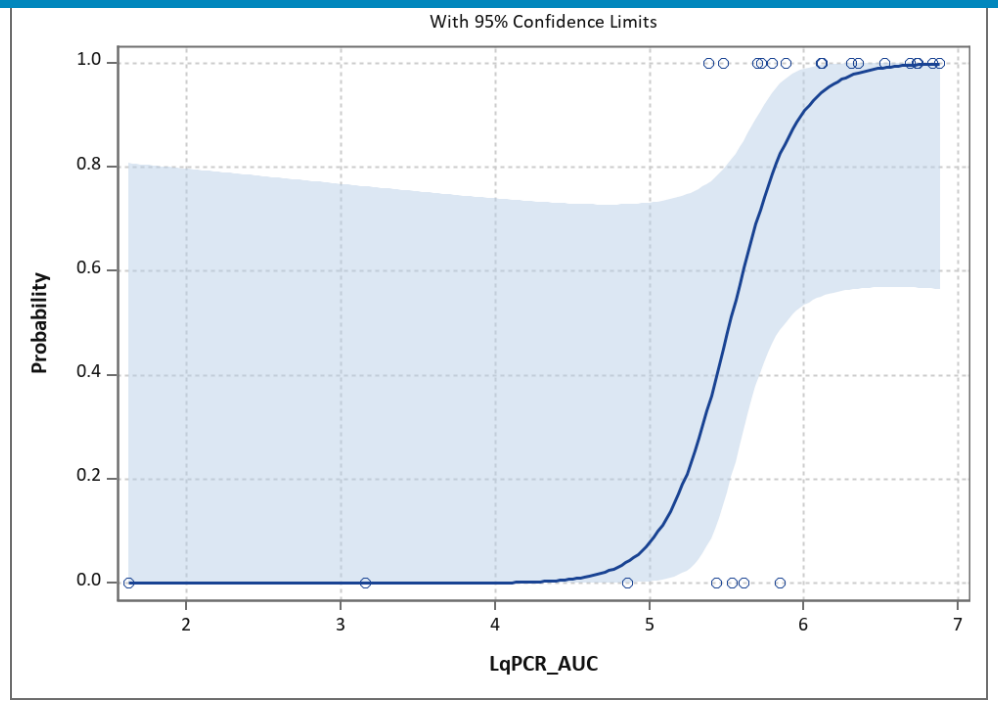
Correlations with Cmax/AUC and Outcome





Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-4.0596	2.8251	2.0649	0.1507
LCmax	1	1.1008	0.6063	3.2967	0.0694

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-26.2121	14.2531	3.3821	0.0659
LqPCR_AUC	1	4.7476	2.5183	3.5541	0.0594

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New Disease Markers in MM/CAR-T: BCMA Correlations?

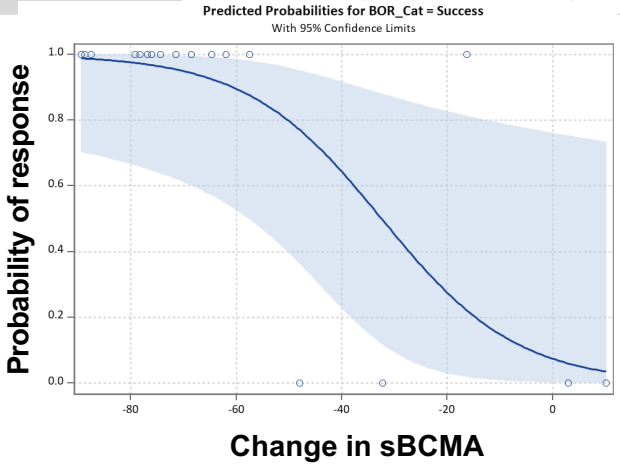
MM cells

- IHC
- Flow
- Transcription

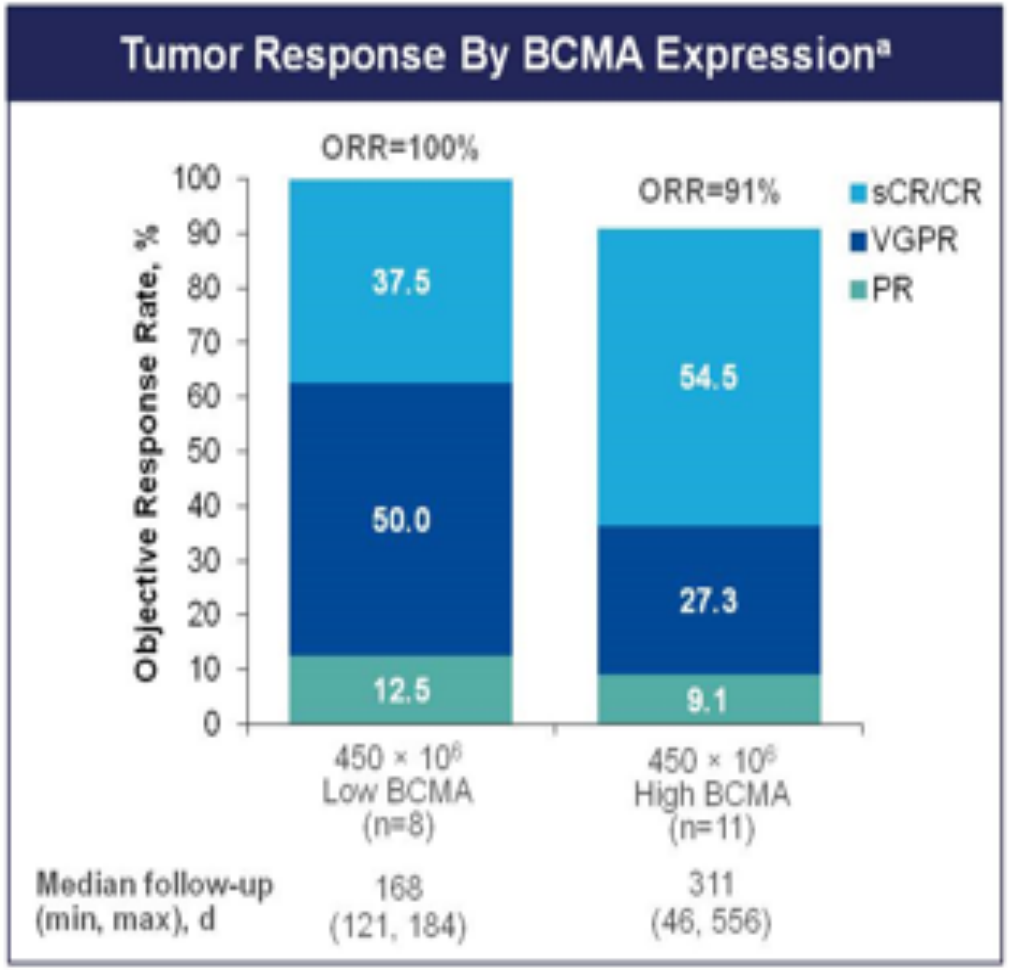
Soluble

- ELIZA
- Luminex

Analysis of Maximum Likelihood Estimates			
Parameter	Estimate	Standard Error	Pr > ChiSq
BCMA Day 0 to 28	-0.0779	0.0362	0.0315



- Statistically significant correlation between decrease in sBCMA in the first 4 weeks and response.
- sBCMA tracks with FLC kinetics



bb2121: Raje et al. ASCO 2018

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New Disease Markers in MM: MRD

- Assessment for residual MM cells in bone marrow

- Increase sensitivity over standard measures of disease burden after treatment (m-protein, FLC, BMPC)
  - Studies indicate complete response of these markers correlate with survival outcomes
  - Most patients relapse in spite of a complete response in these markers

- Methods (bone marrow sample)
  - multiparametric flow cytometry for myeloma-associated markers (MFC) (1:10e5)
  - allele-specific oligonucleotide for IGH rearrangements (ASO)-qPCR (1:10e5)
  - next-generation sequencing of VDJ sequences for rearrangements (NGS) (1:10e6)
    - CTD?

- Correlations with long-term Outcomes:

	Disease status and treatment	N (total)*	MRD-negative patients	Outcomes
Puig et al[33]	GEM2000† and GEM05‡ trials	103 (170)	47%	MRD-negative patients had significantly longer PFS, both in the intensively treated patient group (median 54 months *vs* 27 months; p=0·001) and in the non-intensively treated group (median not reached *vs* 31 months; p=0·029)
Korthals et al[61]	Induction: 2–4 cycles of idarubicin and dexamethasone followed by ASCT	53 (70)	49%	Median EFS in the low-MRD group was significantly longer than in the high-MRD group (35 months *vs* 20 months; p=0·001). Overall survival was significantly longer for the low-MRD group (70 months *vs* 45 months; p=0·04)
Putkonen et al[62]	Patients with multiple myeloma who had achieved a complete response/near to complete response after ASCT or SCT	30 (37)	57%	Low/negative-MRD after ASCT or SCT was a significant predictive factor for the prolongation of PFS (median 70 *vs* 19 months; p=0·003)
Martinez-Sanchez et al[38]	Patients enrolled in the GEM2000* protocol	53 (88)	53%	PFS not reached in MRD-negative patients *vs* 31 months for MRD-positive patients (p=0·001)
Ladetto et al[63]	Four cycles of bortezomib, thalidomide, and dexamethasone consolidation after ASCT	39 (112)	18%	Improved PFS; 100% *vs* 77% at 6 months (grouped by median tumour load as detected by allele-specific oligonucleotide qPCR [p=0·02])
Sarasquete et al[39]	Patients with multiple myeloma who had achieved a complete response after transplantation	24 (32)	29%	Improved PFS for MRD-negative patients (median 34 months *vs* 15 months; p=0·04)
Martinelli et al[64]	Patients who achieved a complete response following ASCT or SCT	44 (50)	27%	MRD-negative patients had a significantly lower relapse rate (41% *vs* 16%; p<0·05) and longer relapse-free survival than MRD-positive patients (median 35 months *vs* 110 months; p<0·005)

Kumar, 2016

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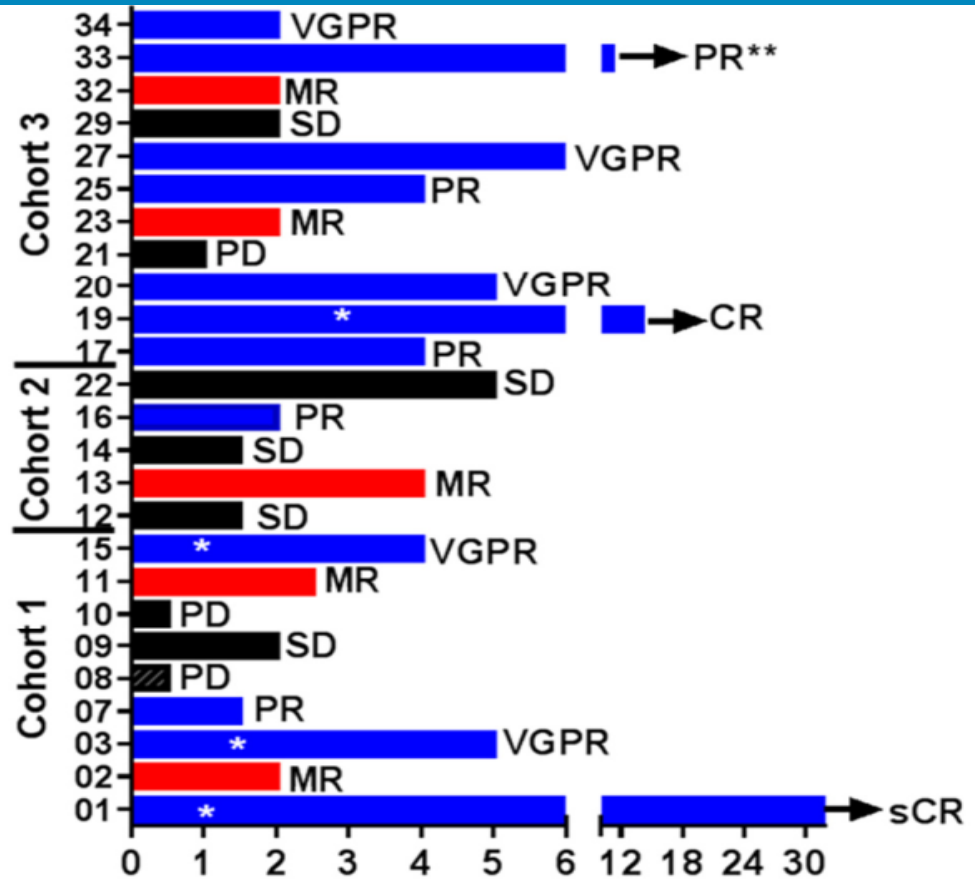
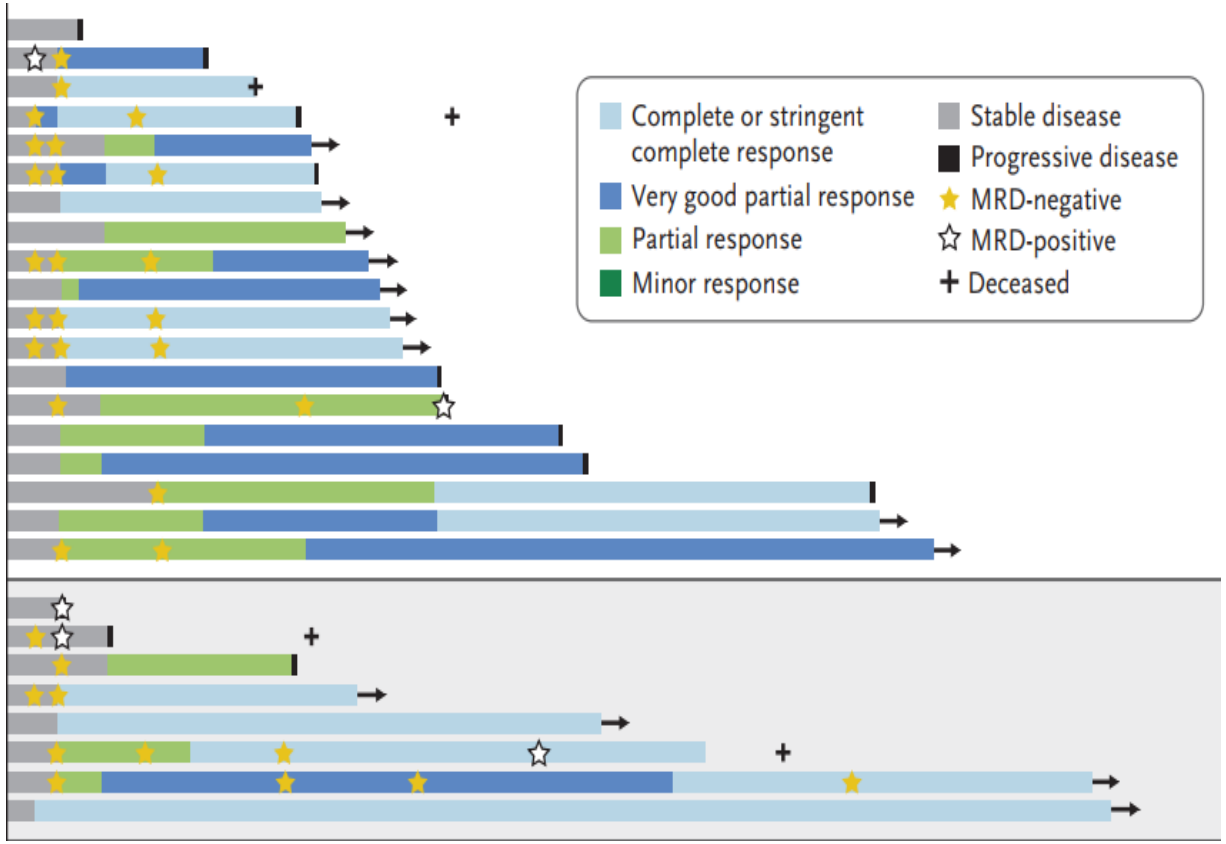
New Disease Markers in MM/CAR-T: MRD Correlations?



CART-BCMA: Cohen et al. J Clin Invest 2019



bb2121: Raje et al. NEJM 2019

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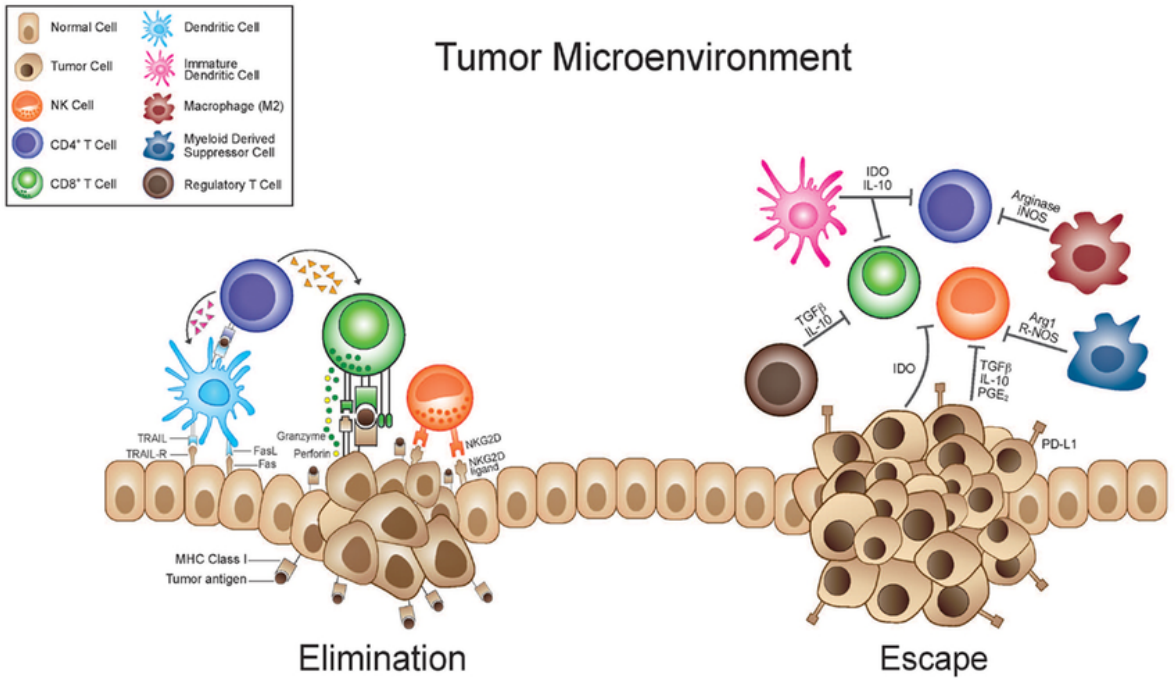
Immunosuppressive Pathways

- **Immunosuppressive tumor microenvironment likely decreases efficacy especially in solid tumors**
  - PD-L1, TGFβ, IL6, IL10, etc…
  - Tregs, MDSC, TAM, etc…
  - poor CAR-T durability



Monjazeb et al. (2013) Frontiers Oncol

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Gene Expression Analysis using Nanostring in CAR-T Cells

Nanostring CAR-T Panel Measures Eight Essential Components of CAR-T Biology

- Optimize CAR-T method development
- Create manufacturing acceptance criteria
- Measure metabolic fitness and persistence
- Monitor post-infusion exhaustion and toxicity

nanoString

Advanced Analysis Modules available for CAR-T Characterization:

- TCR Diversity Score (coming soon)
- Normalization
- Quality Control
- Pathway Analysis
- Cell Profiling
- Differential Expression
- Gene Set Analysis
- Built-in compatibility for Panel-Plus and Protein analysis

From: www.nanostring.com

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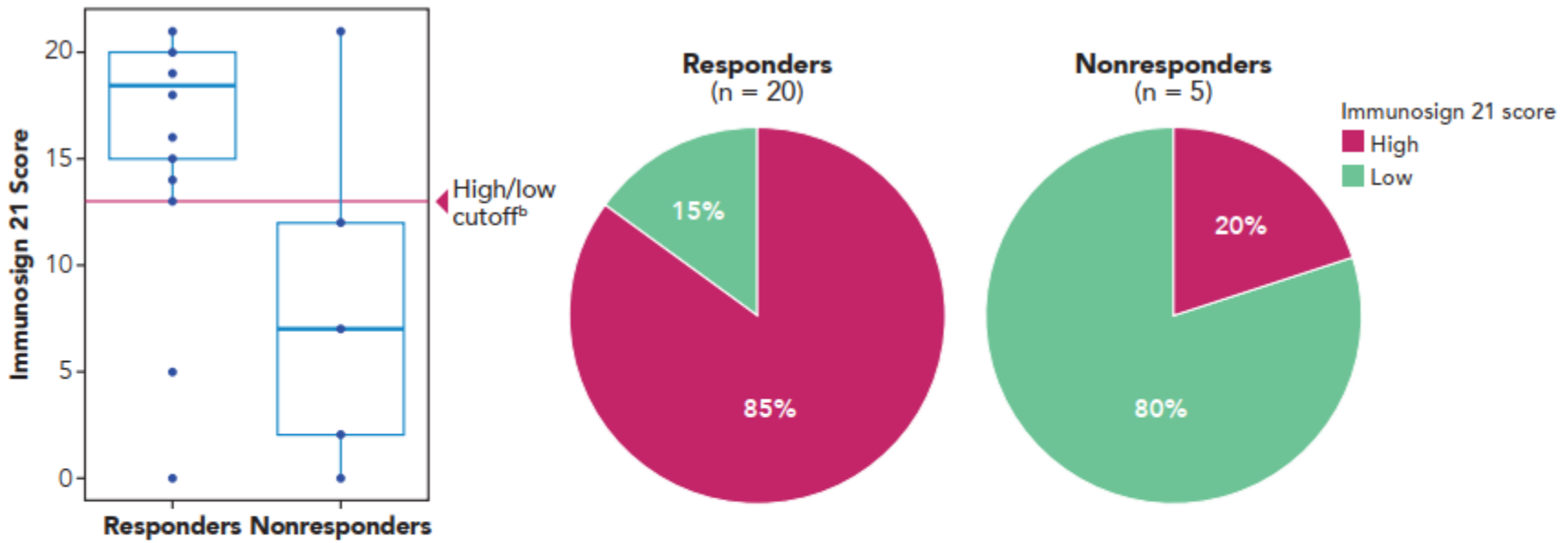
Gene Expression Analysis using Nanostring in DLBCL

High ImmunoSigne21 was Associated with Objective Response

Immunosign 21	
CD3G	STAT4
CD3E	CD3D
GZMK	GZMM
PRF1	CD8A
ICOS	CXCL10
STAT1	IL15
CCR2	CCL2
IRF1	TBX21
GZMA	CXCR3
GZMB	CD69
CXCL11	



[a]This analysis was performed on samples from 25 patients treated with axi-cel with a minimum follow-up of 9 months. One patient subsequently converted from a "nonresponder" to a "responder" at 12-month follow-up.
[b]Cutoff was arbitrarily defined as the 25th percentile of the observed scores among samples.

- A high Immunosign 21 score was associated with objective response at a minimum follow-up of 9 months ($P = .012$; **Figure 5**)
- In a sensitivity analysis, which included the delayed responder, the association between a high Immunosign 21 score and objective response had a $P - .053$

Rossi et al, AACR 2018

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Summary

New Methods are Continually Being Introduced to Evolve CAR-T cells

- The field is nascent with extraordinary results, and advances in genetic engineering and manufacturing techniques allow for extraordinary potential in rationale design to improve CAR-T cells
- P-BCMA-101 incorporates a number of these advances and has been assessed in a clinical trial where it induced high response rates and deep responses in a heavily pretreated r/r MM population, with an excellent safety profile
- In Poseida's clinical trial of P-BCMA-101, %Tscm was strongly correlated with efficacy; proliferative capacity (Cmax and AUM) also correlated with efficacy and durability and strongly support the Tscm hypothesis
- There are significant opportunities in novel biomarker methods to help guide the evolution of the field

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