Immunogenomic approaches to more effective childhood cancer therapies

Kris Bosse, MD
Children’s Hospital of Philadelphia (CHOP)
University of Pennsylvania Perelman School of Medicine
Many Thanks

- **CHOP/Penn**
  - John Maris
  - Samantha Buongervino
  - Maria Lane
  - Sharon Diskin
  - Hakon Hakonarson
  - Kate Krytska
  - Ben Garcia
  - Bruce Pawel
  - Dan Martinez
  - Dimitri Monos
  - And many others

- **SBF-Su2C and Moonshot Projects**
  - Crystal Mackall and Kara Davis
  - Paul Sondel and Ken DeSantes
  - Poul Sorensen and Kirk Schultz
  - Dimiter Dimitrov and Dontcho Jelev
  - Nabil Ahmed and Will Parsons
  - Mike Jensen and Julie Park
  - Michael Taylor, Uri Tabor
  - and Daniel Morgenstern
  - Terry Fry and Lia Gore
  - Nirali Shah and Rosie Kaplan

- **Funding sources for presented work**
  - NIH, SU2C, St. Baldrick’s, ALSF, Evan Foundation, Damon Runyon Cancer Research Foundation, Zymeworks, Tmunity

- **UCSC**
  - David Haussler
  - Sofie Salama
  - Nikolaos Sgourakis

- **PPTC Investigators**
  - Malcolm Smith

- **U of Tubingen**
  - Stefan Stevanovic
  - Daniel Kowalewski
  - Moreno Di Marco
Immunogenomics to Create New Therapies for High-Risk Childhood Cancers: Expanding the targetable cell surfaceome

Class

Cluster of Differentiation

CD19, CD22, CD99, CD276
CD24, CD33, CD123, CD44v6

Adhesion and Motility Molecules

L1CAM, NCAM1
MCAM, ALCAM, CRLF2

Cell Surface Receptors and Transporters

IL13RA2
SLC6A2, CHRNA5, TEM8

Gangliosides

GD2, GD3

ECM-interacting proteins

MSLN, MUC1
STEAP1, CAMKV, PAPPA, DLL3, DLK1

Proteoglycans

CSPG4, GPC2

Receptor Tyrosine Kinases

ERBB2, ALK, FGFR4, ROR1
MET, FLT3

Peptides (oncogenic fusions, etc.)

HLA-A2
NBL peptides

L1CAM, L1 cell adhesion molecule; NCAM1, neural cell adhesion molecule 1; MCAM, melanoma cell adhesion molecule; ALCAM, activated leukocyte cell adhesion molecule; CRLF2, cytokine receptor like factor 2; IL13RA2, interleukin 13 receptor subunit alpha 2; SLC6A2, solute carrier family 6 member 2; CHRNA5, cholinergic receptor nicotinic alpha 5 subunit; TEM8, tumor endothelial marker 8; GD2, GD2 ganglioside; GD3, GD3 ganglioside; MSLN, mesothelin; MUC1, mucin 1 cell surface associated; STEAP1, six transmembrane epithelial antigen of the prostate 1; CAMKV, CaM kinase like vesicle associated; DLL3, delta like canonical notch ligand 3; DLK1, delta like non-canonical notch ligand; PAPPA, pappalysin 1; CSPG4, chondroitin sulfate proteoglycan 4; GPC2, glypican 2; ERRB2, erb-b2 receptor tyrosine kinase 2; ALK, anaplastic lymphoma kinase; FGFR4, fibroblast growth factor receptor 4; ROR1, receptor tyrosine kinase like orphan receptor 1; MET, MET proto-oncogene receptor tyrosine kinase; FLT3, fms related tyrosine kinase 3; NBL, neuroblastoma
Cure rates for childhood cancers have improved dramatically in the latter decades of the last century, but.....

- Treatments are toxic and survivors have life-long disabilities
- Cure rates not improving any longer
- Relapsed pediatric cancer is lethal
Recent credentialing of pediatric cancer immunotherapies

Anti-GD2 monoclonal antibody for neuroblastoma

Anti-CD19 chimeric antigen receptor (CAR) T cells for ALL

Yu, NEJM 2010  Maude, NEJM 2015

Event-free Survival (%)

0 1 2 3 4 5 6
Years since Randomization

P = 0.01

Immunotherapy

Standard therapy

Probability of Event-free Survival

0 3 6 9 12 15 18 21 24
Months since Infusion

Survival rate at 6 mo, 67% (95% CI, 51–88)

Unituxin®
(dinutuximab)
Injection

FDA approved 2015

KYMRIAH™
tisagenlecleucel
Suspension for IV infusion

FDA approved 2017
Rationale for a focused pediatric immunotherapy effort

Vastly different tumors and hosts

• Childhood Cancers
  • Minimal environmental influence
  • Low mutation burdens
  • FDA approved immunotherapies solely synthetic
  • Host immune system naïve
  • Cancer surfaceome reflects developmental origins
    • Lack of targets

• Adult Cancers
  • Major environmental influences
  • High mutation burdens
  • Majority of FDA approved immunotherapies activate adaptive immunity
  • Host immune system exhausted
  • Cancer surfaceome reflects tissue of origin
    • Lack of targets
Neuroblastoma

• Embryonal cancer
  • Misappropriation of normal sympathetic neurodevelopmental pathways
  • Median age of diagnosis = 17 months

• Important pediatric problem
  • 12% of childhood cancer mortality

• Diverse clinical courses
  • Phenotype defined by tumor genomic alterations (MYCN amplification)

Cheung and Dyer, Nat Rev Cancer, 2013
High-risk neuroblastoma has a poor prognosis

- **Low-risk**
  - Observation and/or surgery

- **Intermediate-risk**
  - Surgery
  - Outpatient chemotherapy (2-8 cycles)

- **High-risk**
  - Surgery (1 month)
  - Intensive induction chemotherapy (6 months)
  - MIBG therapy (2 months)
  - Myeloablative chemotherapy with stem cell rescue (Auto-BMT) x 2 (4 months)
  - Radiation therapy (1 month)
  - GD2 immunotherapy (7 months)

MIBG, Metaiodobenzylguanidine; BMT, bone marrow transplant
Discovery and validation of new pediatric cancer cell surface molecules for immune-based therapies

Malone, CF and Stegmaier, K, Cancer Cell 2017

ADC, antibody-drug conjugate; ADCC, antibody-dependent cellular cytotoxicity
Identification of GPC2 as a differentially expressed cell surface molecule in high-risk neuroblastoma

126 high-risk
7,859 samples from 31 unique normal tissues

296 (0.48%) genes with minimum LogFC > 1 and p < 0.01

33 (11%) genes predicted to be membrane associated

9 genes with high absolute RNA expression (FPKM > 50)

(GAP43, SNF8, L1CAM, CHRNA3, CACNG4, GPC2, ZACN, SLC29A4, FSD1)

GPC2

Bosse, Cancer Cell, 2017
GPC2 is a cell surface heparan sulfate proteoglycan

- Glypican (1-6) are cell surface signaling co-receptors
- GPC2 co-receptor unknown
- **GPC2** locus on chromosome 7q
  - 7q copy number gain present in majority of MYCN\(^{\text{wt}}\) high-risk neuroblastomas
- GPC3 being developed by several groups as an immunotherapeutic target in liver cancers

Kurosawa, N et al. 2001, Mythreye, K et al. 2009, Melo, SA et al. 2015, Grobe, K et al. 2015
GPC2 functions as an oncoprotein

*\( p < 0.0001; \) **\( p < 0.001 \)

Bosse, Cancer Cell, 2017
GPC2 is a direct MYCN target gene located on chromosome 7q22

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$; ns, not significant

Bosse, Cancer Cell, 2017
GPC2 is highly expressed on the neuroblastoma stem cell
Development of antibody-based reagents to target GPC2

D3-GPC2-scFv

D3-GPC2-IgG1

D3-GPC2-PBD

*GPC2-expressing neuroblastoma cell line IC$_{50} < 10$ pm

Dimitrov, UPitt, Bosse, Cancer Cell, 2017
D3-GPC2-PBD causes prolonged tumor regression and progression free-survival in the murine NB-1643 PDX model

NB-1643: GPC2\textsuperscript{Hi}, MYCN amplified, ALK mutated, TP53 wild-type

Bosse, Cancer Cell, 2017
D3-GPC2-PBD eradicates locally advanced neuroblastoma PDX models

COG-N-421x: GPC2\textsuperscript{Hi}, \textit{MYCN} amplified, \textit{ALK} wild-type, \textit{TP53} wild-type
The GPC2-directed D3 Fab binds a conformational epitope

<table>
<thead>
<tr>
<th>Protein</th>
<th>BSA (Å²)</th>
<th>Polar bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy chain</td>
<td>765</td>
<td>15</td>
</tr>
<tr>
<td>Light chain</td>
<td>431</td>
<td>4</td>
</tr>
</tbody>
</table>

Jean-Philippe Julien, Sick Kids

Fab, fragment antigen binding; BSA, binding surface area
D3-GPC2-PBD binds tumor specific GPC2 epitopes

GPC2 FL (62 kDa)

5' 1 2 3 4 5 6 7 8 9 10 3' GPC2-001 Tumor

GPC2 Ex5-10 (34 kDa)

5' 5 6 7 8 9 10 3' GPC2-003 Normal tissue

Jean-Philippe Julien, Sick Kids
GPC2 is highly expressed in many human cancers

Cancer cell line encyclopedia (CCLE) data
D3-GPC2-PBD potently eradicates the small cell lung cancer (SCLC) H526 xenograft
GPC2 clinical translation

• GMP-grade ADC manufacturing ongoing
  • Parallel trials in neuroblastoma and SCLC planned

• CAR T cell companies developing GPC2-directed CARs with plans for Phase 1 trials in 2020-21

• Bispecific GPC2-directed therapeutics being developed
  • T cell engagers
  • GPC2 and other neuroblastoma specific cell surface proteins
Multi-omics approach to discovering and validating new cell surface molecules for immune-based therapies

**Surfaceome Generation**

- **Proteomics**
  - Samples: Cell lines (n=12), PDX (n=10), Tumors (n=43)
  - Method: Sucrose gradient ultracentrifugation followed by LC-MS/MS

- **Transcriptomics**
  - Samples: Cell Line (n=40), PDX (n=30), Tumors (n=2,242)
  - Method: RNA-sequencing and microarray

**Prioritization**

- Limited Normal Tissue Expression
  - GTeX, Human Proteome, etc.

**Validation and Functional Studies**

- Immunofluorescence (IF)
- Flow cytometry
- Immunohistochemistry (IHC)*

*Pediatric Cancer and Normal Tissue Microarrays (TMAs)

Amber Weiner

LC-MS/MS; liquid chromatography with tandem mass spectrometry; RNA, ribonucleic acid
DLK1 is highly expressed in a subset of high-risk neuroblastomas.

Patient-derived xenograft (N=10)

Normalized MS Intensity

Protein Rank

MYCN-Amplified
Not Amplified

<table>
<thead>
<tr>
<th>PDX Model</th>
<th>ALK</th>
<th>CD276</th>
<th>GPC2</th>
<th>DLK1</th>
<th>L1CAM</th>
<th>NCAM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>COG-452</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COG-421</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COG-415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB1643</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COG-453</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COG-519</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COG-424</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHLA-79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

normalized iBAQ
n.d. | >5

DLK1, ALK, CD276, GPC2, L1CAM, NCAM1
*DLK1* expression is driven by a super-enhancer element

Identification of a *DLK1* super-enhancer in a subset of neuroblastoma cell lines

Differential H3K27ac signal at *DLK1* locus in neuroblastoma cell lines
ADCT-701 shows potent and specific *in vivo* anti-tumor activity in NBL PDX models expressing DLK1
N-myc Amplification Causes Down-Modulation of MHC Class I Antigen Expression in Neuroblastoma

Immunosurveillance and Survivin-Specific T-Cell Immunity in Children With High-Risk Neuroblastoma

Mark Yarmarkovich

Figure 1. Northern Blot Analysis of RNA Isolated from Human Neuroblastoma Cell Lines

MHC, major histocompatibility complex
Tumor-specific neuroblastoma antigen discovery process

8 PDX NBs
8 primary NBs

Ligandomics

Healthy tissue

DNA/RNA sequencing

Computational

HLA typing

p/MHC binding affinity

Differential Gene expression

Tumor/healthy MHC antigens

Tumor specific antigens

MHC Capture

Peptide elution

Peptide ID

LC/MS/MS

HLA, human leukocyte antigen; p/MHC, peptide-major histocompatibility complex; ID, identification
Tumor-specific antigens discovered in neuroblastoma PDX tumors

8 NB PDX tumors

- 497,501 Peptides
- Binding affinity: NetMHC (affinity <500nM)
- 14,119
- Differential expression: Parent gene expression in 153 NB vs. 1641 normal tissue samples (GTEx)
- 338
- Absence in normal ligandome: Compared to ligandomes of 190 healthy tissues
- 83

4 tumor antigens prioritized and validated

Prioritization criteria:
- MHC/peptide predicted binding affinity
- HLA allele frequency in population
- Differential expression in neuroblastoma
- Absence on MHC in normal tissue
- MHC antigen abundance
- Recurrent across tumors
- Important in neuroblastoma biology

<table>
<thead>
<tr>
<th>Gene</th>
<th>HLA allele</th>
<th>HLA frequency (TCGA)</th>
<th>Abundance rank (percentile)</th>
<th>Peptide-MHC binding affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBPL1</td>
<td>A*02:01</td>
<td>43%</td>
<td>99.4%</td>
<td>4.15nM</td>
</tr>
<tr>
<td>GFRA2</td>
<td>A*02:01</td>
<td>43%</td>
<td>97.6%</td>
<td>22.8nM</td>
</tr>
<tr>
<td>PHOX2B</td>
<td>A*24:02</td>
<td>17%</td>
<td>99.1%</td>
<td>54nM</td>
</tr>
<tr>
<td>PBK</td>
<td>A*24:02</td>
<td>17%</td>
<td>99.7%</td>
<td>23.1nM</td>
</tr>
</tbody>
</table>

Furlan, Lübke, Adameyko, Lallemand, & Ernfors, 2013; Guo et al., 2018; Lee et al., 2016; Z. Li et al., 2019; Marachelian et al., 2017; Raabe et al., 2007
Prioritized antigens are recurrently presented on MHC in neuroblastoma and absent on MHC in healthy tissue (primary tumor validation)

11,587 antigens (5002 genes)

4373 antigens (2812 genes) previously unobserved in 190 normal tissues

Single gene ontology (GO) enrichment term: sympathetic nervous system development
Tumor antigens are derived from parent genes under the control of neuroblastoma core regulatory circuit lineage-restricted oncoproteins

Nate Kendsersky
Wang et. al, Submitted 2019
Durbin et. al, Nature Genetics, 2018
Decaesteker et al., Nat. Comms., 2018
Boeva et al, Nature Genetics, 2017
Groningen et al., Nature Genetics, 2017
Development of scFv binders to the peptide-MHC complex

scFv (single-chain antibody binder) library ($10^{10}$ variants)

Removal of non-specific MHC binders

Enrichment with tumor pMHC 3x

Depletion with decoy pMHC magnetic beads

ELISA matched vs. decoy pMHC

PHOX2B

IGFBPL1

scFv, single chain variable fragment; ELISA, enzyme-linked immunosorbant assay
F11 CAR targeting IGFBPL1 shows specific and potent tumor cell killing

Second generation CAR T construct:

\[
\text{CD8a leader} \quad \text{scFv Heavy} \quad \text{Linker} \quad \text{scFv Light} \quad \text{CD8 Hinge&TM} \quad \text{4-1BB} \quad \text{CD3-zeta}
\]

F11 CAR: anti-IGFBPL1/HLA-A2

Transduced primary CD8 cell killing assay

2:1 E:T

IGFBPL1 CAR T cell

IGFBPL1 TCR

Untreated
Conclusions

• Many lineage restricted genes critical in sympathetic nervous system (and neuroblastoma) development may serve as optimal immunotherapeutic targets
  • Native cell surface molecules
  • Peptides presented via MHC

• Multiple immunotherapeutic strategies being developed to therapeutically target these molecules
  • Biomarker-defined clinical trials are required to show safety and efficacy in children
Immunotherapies for Pediatric Cancer: Current Status and Future Prospects (with an emphasis on Targets)

Pediatric Oncology Subcommittee of the Oncologic Drug Advisory Committee, FDA White Oak Campus, June 20, 2019

Crystal L Mackall MD
Ernest and Amelia Gallo Family Professor of Pediatrics and Medicine, Stanford University
Director, Stanford Center for Cancer Cell Therapy
Director, Parker Institute for Cancer Immunotherapy @ Stanford
Associate Director, Stanford Cancer Institute
Immunotherapy: “A Revolution in Cancer Therapy” Driven Largely by Success with Checkpoint Blockade

What about childhood cancers?

Jim Allison and Takira Honjo
2018 Nobel Prize in Medicine

Drake et al, Nat Rev Clin Onc 2014
Responsiveness to Checkpoint Blockade Varies Widely; Only a Fraction of Cancer Patients Benefit

Lawrence et al, Nature 2013

Genetically Quiet
“Checkpoint Unresponsive”

High Mutational Burden
“Checkpoint Responsive”
A Phase I Trial of Ipilimumab (anti-CTLA4) in Pediatric Patients with Cancer

- 30 children enrolled
- Grade III/IV Immune related adverse events
  - Colitis (3), transaminitis (2), hypophysitis (1)
  - Pancreatitis (1), thyroiditis (1)
- 11 Metastatic Melanoma
  - 0 objective responses
  - Compared to ~8-15% response rate in adult melanoma
  - Pediatric melanomas have lower mutation rates
- 19 non-melanoma
  - 0 objective responses
- ? Modest survival benefit in patients with immune related adverse events

ADVL1412: Initial Results of A Phase I/II Study of Nivolumab (anti-PD1) ± Ipilimumab in Pediatric Patients with Relapsed/Refractory Solid Tumors-A Children’s Oncology Group Study (ASCO, 2018)

- Phase I portion reveals tolerability of nivolumab in children as a single agent at adult RP2D and combination nivo/ipi at adult RP2Ds

- Single agent nivolumab:
  - Ewing sarcoma: 0/10 objective responses
  - Osteosarcoma: 0/10 objective responses
  - Rhabdomyosarcoma: 0/10 objective responses
  - Measurable neuroblastoma: 0/10 objective responses
  - Hodgkin’s disease: 3/11 objective responses

- Ipilimumab (1 mg/kg) plus nivolumab (3 mg/kg)
  - Sporadic responses

*Checkpoint inhibition as a single therapeutic maneuver has limited impact in sporadic childhood cancers*
Children with Ultra-hypermutant Cancers Due to Biallelic Mismatch Repair Respond to PD-1 Inhibition

Germline Bi-allelic Mismatch Repair Deficiency

Sibling #1 ~24,000 mutations/exome

Sibling #2 ~22,000 mutations/exome

Campbell, Cell 2017

Bouffet, J Clin Onc 2016
Many Unanswered Questions Remain Regarding the Potential Role for Checkpoint Inhibition in Pediatric Cancers

- Would combination checkpoint therapy (anti-CTLA4 + anti-PD1) manifest significant response rates in sporadic pediatric cancers stratified as “hypermutant” (>10 mutations/Mb)?
  - A clinical trial testing this hypothesis will be launched in the Pediatric Cancer Immunotherapy Trials Network in the coming months

- Can novel combinations of immunotherapies induce responses in sporadic pediatric cancers?
  - Promising results in neuroblastoma models with irradiation plus anti-GD2 plus anti-PD1 (Sondel lab)
  - Promising results with chemo in combination anti-CD40 plus anti-PD-1 in pancreatic cancer, another “low immunogenicity tumor” (Vonderheide et al, AACR, 2019)
  - There are hundreds of potential combinations….and the RACE for Kids Act will make many more of these agents available for study… the pediatric community can’t study them all…which to prioritize?
    - Combination that demonstrate efficacy in preclinical models that mirror the low mutation burden of pediatric solid tumors in an intact immune system…great need to develop and credential such model systems
    - Combinations that demonstrate efficacy in adult “low immunogenicity” tumors…

- What about novel checkpoints?
CD47 Serves as a "Checkpoint" For Macrophages; Anti-CD47 Blockade Plus Dinutuximab Demonstrates Impressive Synergy in Preclinical Models (Majzner, AACR, 2019)

Dinutuximab upregulates the calreticulin "Eat-Me" signal on neuroblastoma.

Day 1 of Therapy

Day 21 of Therapy

http://med.stanford.edu/majetilab/cd47.html
Distinct Classes of Immunotherapies Based Upon Inherent “Tumor Immunogenicity”

**Immune Response Amplifiers**

- checkpoint inhibitors
- agonist checkpoints
- innate immune activators (e.g. TLR agonists, Sting agonists CpGs, anti-CD40, CD47 blockade)
- MDSC antagonists (CSF1R inhibitors, CXCR2 inhibitors, PI3kγ inhibitors)
- cytokines (e.g. IL-12)
- oncolytic viruses
- tumor vaccines

**Immune Response Initiators: Synthetic Immunotherapies**

- monoclonal antibodies
- bispecific antibodies
- T cells expressing **chimeric antigen receptors**
- T cells expressing affinity matured T cell receptors
Engineered T Cells: T Cell Receptors vs. Chimeric Antigen Receptors

• recognize processed peptides (intracellular or cell surface antigen)
• MHC restricted
• requires co-stimulation from APC or tumor

• recognize intact cell surface antigens
• non-MHC restricted
• costimulatory signal provided coincident with antigen recognition
CD19-CAR Therapy (CTL019, tisagenlecleucel, KYMRIAH): A Watershed Moment

Emily Received CD19-CAR T Cells
Age: 6 yrs

Spring 2012

August 2017

HEALTH

F.D.A. Panel Recommends Approval for Gene-Altering Leukemia Treatment

By DENISE GRADY JULY 12, 2017

✓ First FDA approved cell therapy for the treatment of cancer
✓ First gene therapy approved in the United States
✓ Unusual developmental path: approval in children prior to adults: Still no CD19-CAR therapy approved in US for adults >26 years of ago
✓ First therapy with an outcome based payment model
✓ Trial soon to launch to test Kymriah for MRD+ disease following consolidation in HR-ALL

Emily Whitehead, 12, and her parents, Tom and Kari Whitehead, appeared at an F.D.A. hearing on Tuesday about a treatment for leukemia that had saved Emily’s life. © V. J. Knapik/For The New York Times
Watershed (Oxford): An event or period marking a turning point in a situation

Pre-CD19-CAR

✓ Cell therapy will never work, how could you commercialize it?
✓ Bispecific antibodies do the same thing, why do we need cells?
✓ Autologous products from cancer patients won’t work, they’re too immunosuppressed…
✓ It will take too long to make the product, these are aggressive diseases
✓ It’s too darn expensive

Present Day

✓ It’s too darn expensive, how can we lower the price?
  ▪ $475,000 USD for Kymriah/B-ALL: Highest “sticker price” of any cancer therapy
  ▪ $373,000 USD for Yescarta/DLBCL
  ▪ $373,000 USD for Kymriah/DLBCL
✓ How can we scale up to make products for all patients who need it?
✓ Autologous or allogeneic?
✓ Could it really work for solid tumors?
Scientific Challenges Revealed by Experience in B-ALL:
*Intent-to-treat CR 66%, CR after infusion 81% with ~50% EFS, Sustained Remission Intent-to-Treat ~30%*

Maude et al, NEJM 2018

Major Challenges:
- Manufacturing issues (delays, failures)
- Toxicity: supportive care improving
- Acquired Resistance: Antigen loss escape
  - 15/16 relapses tested were CD19neg

---

![Graph showing event-free and overall survival](image)
CD22-CAR Induces a Similarly High Response Rate in B-ALL as the CD19-CAR....Equal Efficacy in CD19neg Patients Following Previous CD19-CAR

Fry et al Nat Med, 2018 and updated data from Nirali Shah, NCI Pediatric Oncology Branch
Resistance: Antigen Lo Relapse Commonly Occurs After CD22-CAR
Options for Simultaneous Targeting of Two Antigens by CAR-T cells

- **Co-administration**
  - Under study at CHOP

- **Co-expression**
  - Two vectors (Cotransduction)
  - One Bicistronic vector
  - Under study at Seattle Children’s
  - Soon to open at NCI and Children’s Colorado

- **Bivalent-Bispecific Receptor (aka TanCAR)**
  - Open at Stanford and NCI for Children and Stanford for adults
CD19/22 Bispecific CAR Trials at Stanford Reveal Evidence for Clinical Activity and Acceptable Toxicity

- Two trials of CD19/22-bispecific CAR at Stanford underway: Pediatric and Adult
- Relapsed refractory B cell malignancies
  - Primary objectives: Safety and Feasibility
  - Secondary objectives:
    - Response rate
    - What fraction of patients are CD19neg at relapse?
- Early results presented at American Society of Hematology, 2018
  - Significant Clinical Activity
  - Acceptable toxicity
- No CD19 negative escape observed thus far

Ongoing CR following CD19/22-CAR in a patient with lymphomatous B-ALL
Challenges and Opportunities Facing CAR-T Cell Therapy for Solid Tumors

**Challenges**

- Low/Heterogeneous Antigen Expression
- T cell exhaustion (intrinsic T cell dysfunction)
- Suppressive microenvironment (extrinsic T cell dysfunction)
- Insufficient tumor trafficking?

**Opportunity?**

- Epitope spreading could be robust in tumors with high neoantigen levels

*Juntilla, Nature, 2013*
Candidate Cell Surface Targets on Pediatric Solid Tumors With High Differential Expression

- **GD2**
  - Medulloblastoma Group 3, 4
  - Neuroblastoma
- **PAPP-A**
  - ATRT
  - Medulloblastoma
  - Sarcomas
  - Neuroblastoma
  - Some AML
- **B7H3**
  - Ewing Sarcoma
- **GPC2**
  - Neuroblastoma Group 3, 4
  - Neuroblastoma
- **SU-DIPG6**
  - Neuroblastoma
  - Osteosarcoma
- **SU-DIPG13**
  - Neuroblastoma
  - Osteosarcoma

**References**
- Bosse, Cancer Cell, 2017
- Mount, Nat Med 2018
- Majzner, CCR, 2019
- Theruvath, In preparation
- Heitzeneder, JNCI, 2019
Diffuse Intrinsic Pontine Glioma (DIPG): A Devastating Childhood Cancer Highly Overexpresses the GD2 Ganglioside

Now molecularly defined (WHO) as **Diffuse Midline Glioma** by H3K27M mutation

Histone mutation drives widespread hypomethylation and increased transcription

Mackay et al, Cancer Cell 2017

Mount/Majzner, Nature Med, 2018
Intravenous Administration of An “Optimized” GD2-CAR Shows Potent Activity in Murine Models of K27M DIPG and Spares Normal Brain Tissue

Early deaths in a small fraction associated with ventricular enlargement, likely due to tumor swelling

Mount/Majzner, Nature Med, 2018
Neurons in the Tumor Microenvironment are Spared by the GD2-CAR T Cells, Likely Due to Differential Surface Expression

Mount/Majzner, Nature Med, 2018
Clinical Trial of GD2-CAR T cells for Diffuse Intrinsic Pontine Glioma Planned at Stanford

- Single institution, investigator-initiated trial
- Eligibility: 3 months from completion of upfront XRT or earlier recurrence, no corticosteroids, no thalamic/cerebellar involvement, no bulky disease
- Autologous GD2.BBz.iCasp9-CAR T cells (1e6/kg, 3e6/kg, 1e7/kg) following cyclophosphamide/fludarabine conditioning regimen
- Careful monitoring for increased intracerebral pressure
- In the event of unacceptable toxicity
  - Supportive care for elevated ICP
  - Consider dasatinib to reversibly suppress CARs
  - Consider AP1903 to activate the suicide switch
Many Pediatric Solid Tumors Overexpress B7H3 and Regress Following B7H3-CAR T cells in Preclinical Models (Majzner, Clin Can Res 2019)
Summary

- The surfaceome of human cancers is incompletely cataloged.
  - Some solid tumors express high levels of surface antigens amenable to CAR therapy; these deserve prioritization for clinical trials of CARs for solid tumors.
  - Tumor biology drives cell surface phenotype.

- GD2 ganglioside
  - H3K27M diffuse midline gliomas, devastating and incurable cancers of childhood, express ultra-high GD2 levels and GD2-CARs induce impressive regression in preclinical models.
  - In a murine model where GD2 is known to be expressed on the brain, we see no evidence of on-target neurotoxicity, consistent with a therapeutic window for GD2 between tumor and normal tissue.
  - Several clinical trials of GD2-CAR have been conducted with no evidence for neurotoxicity (peripheral or central), consistent with a therapeutic window.

- Several cell surface targets are differentially expressed in pediatric solid tumors that serve as candidates for CAR targets.

- Much more clinical work is needed to better understand the therapeutic “windows” available to CAR T cells.
CAR-T Cell Prototype: 2019

Anticipate clinical testing of more complex and sophisticated CARs in the next 5 years

- Bispecific CARs (OR gates)
- Trispecific CARs (OR gates)
- Quad-specific CARs (OR gates)
- Regulatable CARs (small molecule on or off)
- "Universal" CARs
  - Antibody or other protein administered activates CAR
  - Switch antibody in event of antigen escape
- AND Gate CARs (increase specificity for antigen groups)
- Switch Receptor CARs
  - Turns an inhibitory signal in the tumor microenvironment into an activating signal
- Exhaustion-resistant CARs
- CARs engineered to recognized low antigen density
- CARs that make their own growth factors
  - No lymphodepletion, better persistence?
- Allogeneic off-the-shelf CAR
- γδ-CAR, NKT-CAR
- ........

Clinical trials now or soon

CD19
CD22
19/22
GD2
B7H3
L1CAM
Her2
CD33
CD123
IL13Ra2
CD30
ROR1
CD20
EGFRvIII
BCMA
Mesothelin
PSMA
GPC3
Muc1
....

2nd generation CAR signaling

Pediatric relevant targets
Future Challenges to Developing CAR Therapies: 
Scientific and Manufacturing Hurdles

Shultz and Mackall, Sci Trans Med, 2019
FDA Approvals: 3 in 2017/2018
- Kymriah for Pediatric/Young Adult B-ALL
- YesCarta for Adult Large B Cell Lymphoma
- Kymriah for Adult Large B Cell Lymphoma

Yu, Nature Reviews Drug Discovery, May 2018
- cell therapy represents the largest number of agents under study in the immune oncology space
- 1,011 active agents in global cell therapy development pipeline (25% increase in one year)
- 568 CAR T cell agents in clinical studies
  - 130 CD19 CARs under study
  - 36 BCMA CARs under study
- 439 agents in US, 305 agents in China (74% of total)
- 1216 clinical trials of cancer cell therapies 1993-2019; 762 active studies
- ~50% of trials in solid tumors

FDA Statement: January 15, 2019
“….We anticipate that by 2020 we will be receiving more than 200 INDs per year, building upon our total of more than 800 active cell-based or directly administered gene therapy INDs currently on file with the FDA. And by 2025, we predict that the FDA will be approving 10 to 20 cell and gene therapy products a year based on an assessment of the current pipeline and the clinical success rates of these products….“
2019: Adoptive Cell Therapy Immuno-Oncology Landscape

Source: company filings and presentations; industry research
Evolving Models for Manufacturing: Off-the-Shelf, Centralized, Distributed

- **Off-the-Shelf**
  - The vision is that hundreds or thousands of products could be made from a “super donor”
  - Requires extensive engineering to prevent GVHD and graft rejection
  - Benefits are lower cost and consistent products
  - Significant scientific progress needed to realize the vision

- **Centralized Manufacturing**
  - Current FDA and EMA approved CAR T cell products are manufactured in a centralized commercial manufacturing plant.
  - Centralized manufacturing is currently very costly
  - Immense pressure to bring costs down
  - Tighter margins in the future will likely de-incentivize the private sector from developing CARs for rare indications, such as pediatric cancers

- **Distributed manufacturing**
  - Hospitals would manufacture individualized products using automated platforms
  - Regulatory approval would apply to the construct/process
  - Akin to bone marrow transplantation at specialized medical centers
  - May enable increased availability for rare disease indications
Rapidly Evolving Technologies for Manufacturing

“Miltenyi Prodigy” distributed manufacturing model could enable production within hospitals

- One Prodigy one pts product
- Single operator - multiple products
- Validated barcode system
- Each unit operates independently

“Lonza’s” cocoon closed manufacturing system could allow for unprecedented scale-up
Conclusions

- CAR T cells made their debut in pediatric oncology and drove a paradigm-shift in the field of cancer cell therapy and gene therapy.
- Many manufacturing challenges and scientific challenges remain. But, the science and emerging technology in the field is very robust.
- FDA, academic medical centers and private sector are betting that this field that will grow dramatically in the next 5-10 years.
- Continued positive clinical results will continue to drive technological advances and decreased cost of goods which will, in turn, drive costs down and increase accessibility in coming years.
- Children with cancer could continue to benefit from these advances, especially if the distributed manufacturing model is adopted by regulatory agencies and leading medical centers.
Acknowledgements

Mackall Lab
- Rachel Lynn
- Evan Weber
- Elena Sotillo
- Robbie Majzner
- Sabine Heitzeneder
- Johanna Theruvath
- Dorota Klytz
- Peng Xu
- Meena Malipotlalla
- Meena Kadapakkam

Stanford Clinical
- Rebecca Richards
- Bita Sahaf
- Diane Tseng
- Louai Labanieh
- Justin Arredondo-Guerrero
- Zina Good
- Hima Abunathan
- Jake Lattin
- David Miklos
- Kara Davis
- Liora Shultz
- Lori Muffly
- Nash Hossain
- Katie Kong
- Sharon Mavroukakis
- Tina Baggott
- Matt Abramian
- Steve Feldman
- Shabnum Patel
- Matt Abramian
- Anne Marcy

Stanford Collaborators
- Howard Chang
- Ansu Satpathy
- David Gennert
- Michelle Monje
- Christopher Monje

NCI POB
- Terry Fry
- Nirali Shah
- Haiying Qin
- Sneha Ramakrishna

St. Baldrick’s-SU2C Team
- John Maris
- Chris Bosse
- Poul Sorensen