National Cancer Institute

Society of Immunotherapy in Cancer

October 25, 2012, North Bethesda, MD

Antibody-Based Therapeutics in Cancer

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DCTD

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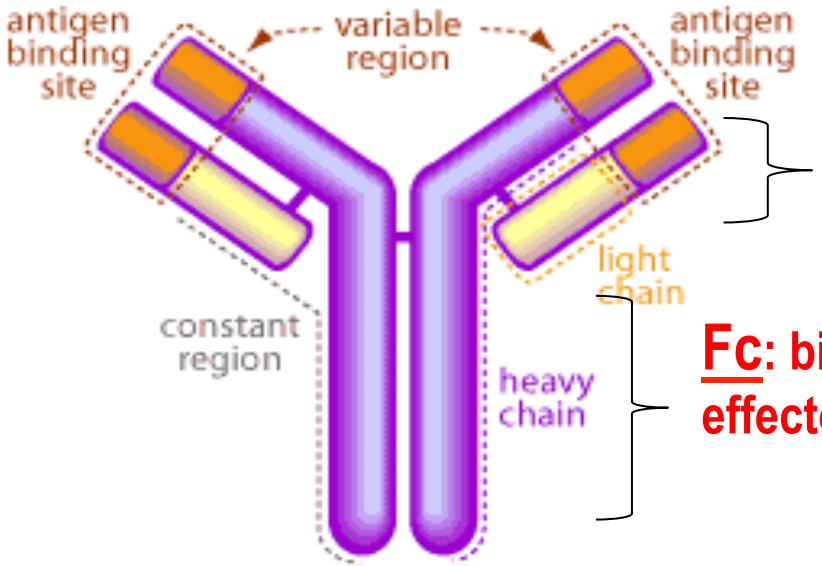
OUTLINE

- Basic concepts
  - Immunology of Ab
  - Types of Ab-based therapies
- Naked monoclonal antibody (mAb)
  - Mechanisms of action through Fv and Fc
  - Approaches to optimization
- Novel Ab constructs to expand the “effectors”
  - Redirecting T- cells to cancer cells
  - Redirect drug payloads to cancer (Ab-drug conjugates)

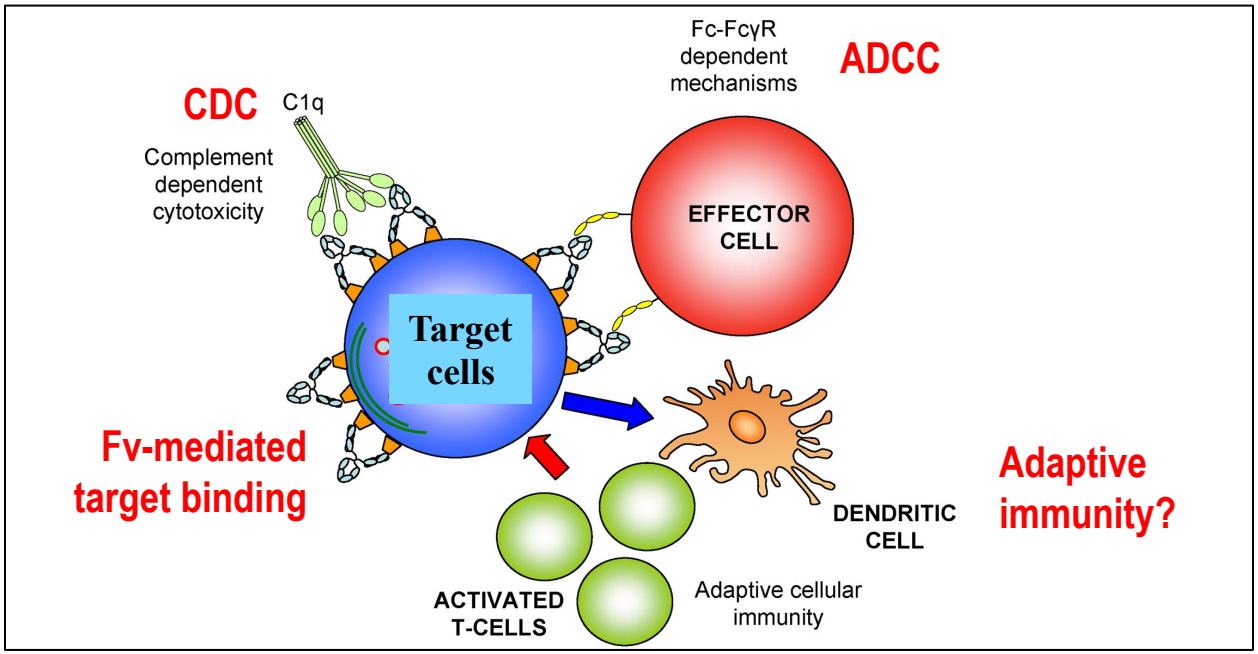
Basic structure of IgG

- **Bivalent monomer:**
  - 2 Heavy Chains:
    - *Variable (V_H) + constant (C_H)* regions
  - 2 Light chains: V_L+ C_L
- 4 subclasses:
  - IgG1, 2, 3 and 4



Fv: antigen binding site

Fc: binding to host effector cells



Key features of antibodies

- **Through Fv, diverse and exquisite specificities against target antigens, or epitopes** (⇐ Immunoglobulin variable gene rearrangement in B cells and hypermutation)

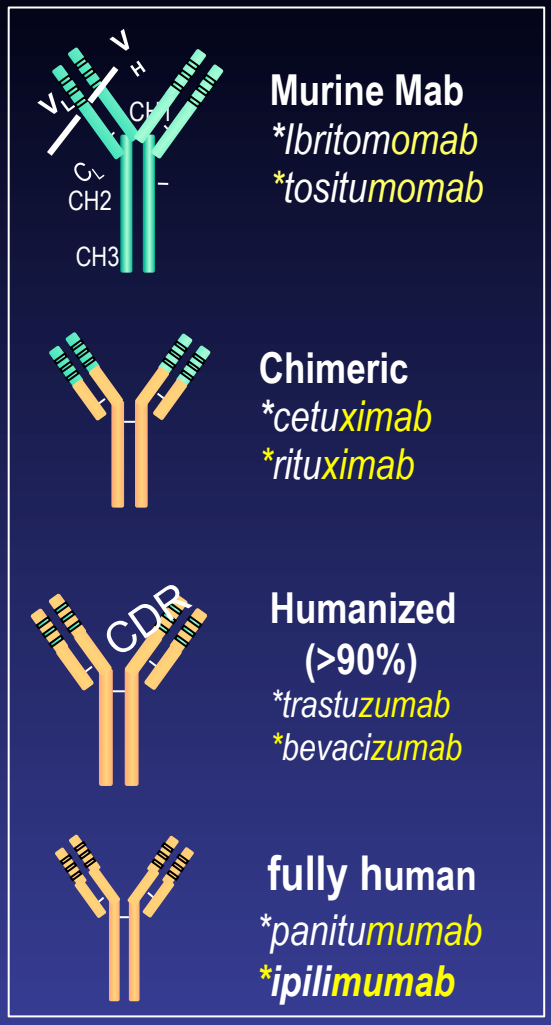
- **Through Fc, ability to engage host immune components to targets** (complement, NK cells, macrophages, neutrophils, DC)

- **Distinct MOA from cellular immunity**
  - Unlike TCR, Abs recognize unprocessed antigens independent of MHC

- ***Potential as a therapeutic platform:***
  - Cell-free protein product
  - Long half-lives (150 KD MW), unlikely to have PK interactions with small molecules
  - Vast repertoire of B-cells with unique Fv regions for targets of interest
  - Modifiable for variable sizes and novel constructs: *full IgG, Fab, scFv ….bispecific Ab, drug conjugates*

History of monoclonal antibodies (mAb) for therapy

- **1975:** **First murine MAb from hybridoma** (Kohler and Milstein, Nature)
- **1982:** **Anti-idiotypic mAb against lymphoma** (Millar and Maloney)
- **1980's-90's:** **Humanization of murine Abs**
  - Recombinant chimeric Ab ('84)
  - CDR grafting → humanized Ab ('86)
- **1998**: **Fully human Mab**:
  - *XenoMouse*
  - *Phage scFv library*
- ***Novel construct:*** Bispecific; Ab-drug-conjugate …



➢ **1997- 2012: > 20 mAbs approved for cancer therapy**

Approved agents and New progress

Targets	Approved	New/emerging (a partial list)
• Tumor or stromal cell growth/survival factors	*Cetuximab, Panitumumab *Trastuzumab, Pertuzumab * Bevacizumab, VEGF-TRAP	• Erb3, c-MET, HGF • FGF, Angiopoeitin
• Tumor Ag (action through effectors)	*Rituximab, *Ofatumumab; *Alemtuzumab	• Ch14.18 (anti-GD2)
• Host immunity (immunemodulator)	*Ipilimumab	• PD1/PD-L1; CD40; OX40, 4-1BB • CD137, CD25
• Ab-cytotoxic conjugate	*Ibritumomab (zevalin) *Toxitumomab (baxxar); *SGN-35	• Trastuzumab-DM1 • CD19, CD22, CD56 • PSMA, EphA2, Integrin,
• Bispecific mAb:	*Catumaxomab (EPCAM xCD3 x FcR)	• Blinatumomab (CD19xCD3 BiTE) • EpCAM xCD3 BiTE

Unlabeled Full IgG Antibody Therapies

» Mechanism of action

» Strategies of optimization

Mechanism of Action of mAbs

Mediated by Fv binding to targets

- **Block target signaling**
  **EGFR, HER2*
  **CTLA4*
- **Induce program cell death**
  **Rituximab*
- **Stimulate target signaling**
  **CD40, OX40 on T cell*
  **TRAIL-DR5 on tumor cells*

Mediated by Fc binding with innate host immune system

- **ADCC** (Fc-FcR meditated)
  **Rituximab*
  **Others (IgG1 mAb against HER2, EGFR)*
- **CDC** (Fc-complement)
  **Campath-1H*

Adaptive Immunity through FcR on APC?

**Reported with rituximab and trastuzumab*

Not all MOA apply to all mAbs. Relevance to efficacy may differ by the target, the clinical setting and the agent

Attempts to improve the efficacy of full IgG mAbs

- **Optimize the Ag-binding site**
- **Enhance the Fc mediated effector functions**
  - **Fc modulation**
  - **Combination with immune cytokines**

Optimize the Ag-binding site (Fv) … *for the right epitopes and affinities (1)*

Hundreds of unique mAbs can be created against a single target molecule, that recognize different epitopes, with variable affinities … *not all Ab drugs for a target are created equal*

The antigen-bindings sites may be selected or optimized for desired features:

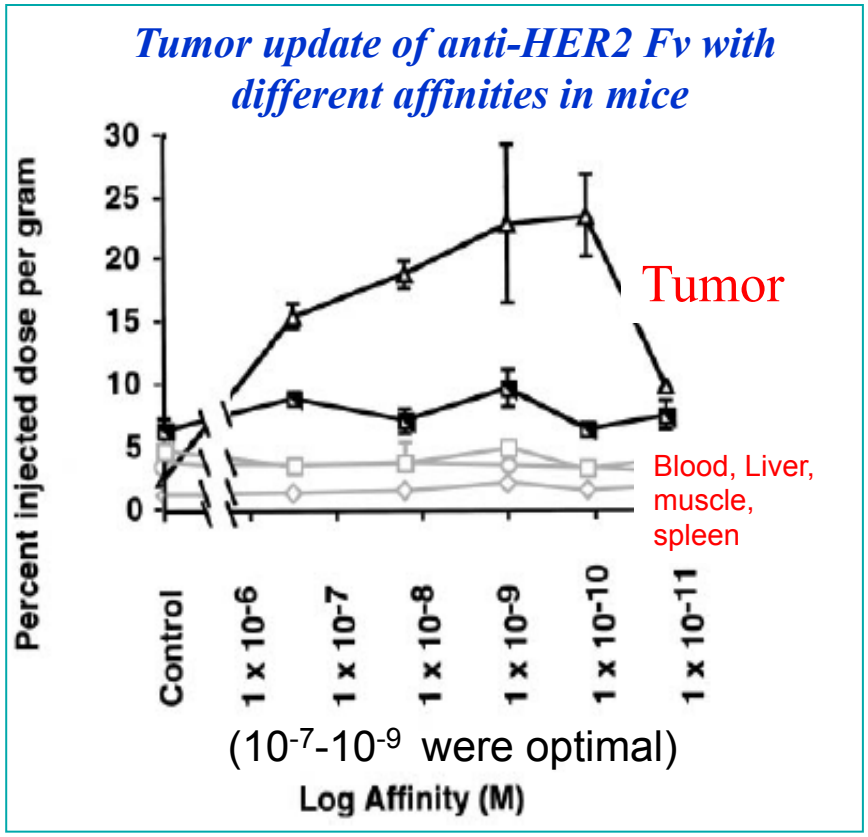
- **Different mechanisms of antitumor effect**:
  - *Rituximab → GA101;
  - * Trastuzumab → pertuzumab
- **Agonist vs. Antagonist**
  - *CD40 agonist (**CP-870,893)** - as immunotherapy *
  - *CD40 antagonist **(CHIR-12.12) -** as tumor - targeting agent (e.g. CLL)
- **Different affinity or avidity …**

Optimize Ag-binding site (Fv) – for the right epitopes and affinities (2)

- ***Is higher affinity better?***
  - Higher affinity has better target engagement and ADCC
  - ***However***, too high an affinity is not always desirable
    - Lower penetration in tumor
    - Excessive activation of effector cells (some anti-CD3 mAbs)

Affinity should be optimized for different settings

- solid vs. "liquid" tumors
- Tumor vs. host immune cell targets



Adams et al, Ca Res, 2001

Enhance the host effector cell function (including ADCC)

Is ADCC a MOA of antitumor effects in patients?

- Direct *in vivo* evidence of ADCC is not available. However, there are indirect evidences:

  – *Preclinical:*

    - **Knockout of FcγR gene in mice or mutation of Ab Fc can reduce antitumor effects of anti-CD20 and anti-HER2 antibodies**

  – *Clinical*:

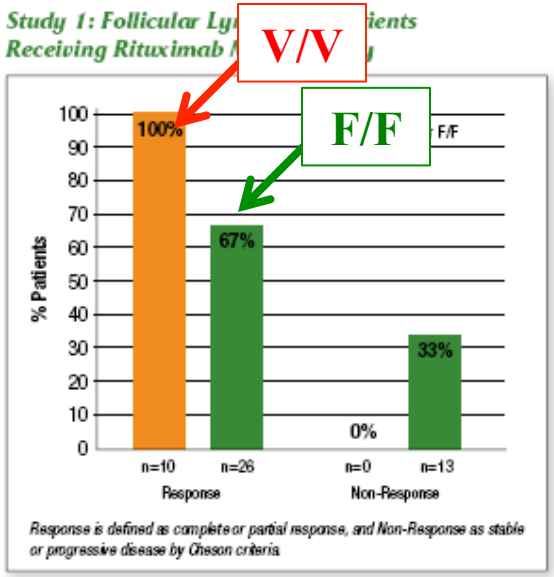
    - **In patients, polymorphism of the host FcR receptor affected activity of rituximab in follicular lymphoma …**

Host Fc Receptor polymorphism and mAb Activity

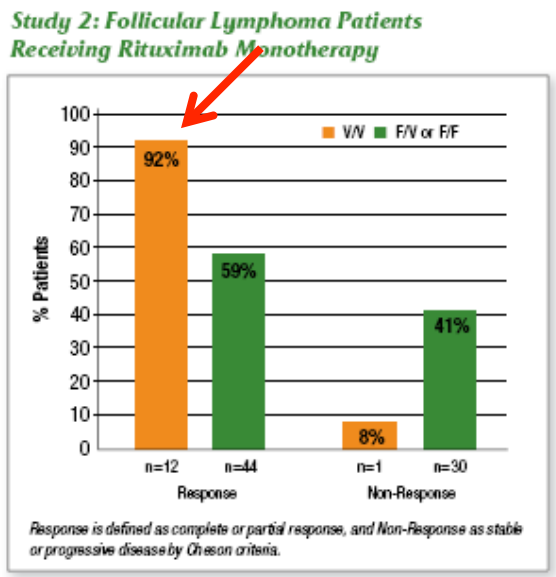
FcγRIIIA polymorphism (4985G>T) with phenylalanine (F) to valine (V) substitution at aa position 158

- **158 V/V** has greater affinity Fc compared to **158 F/F** → greater ADCC in vitro

- **Rituximab in FL: 158 V/V Predicted better response than F/F**
  - **92-100%** *vs.* **53-64%**



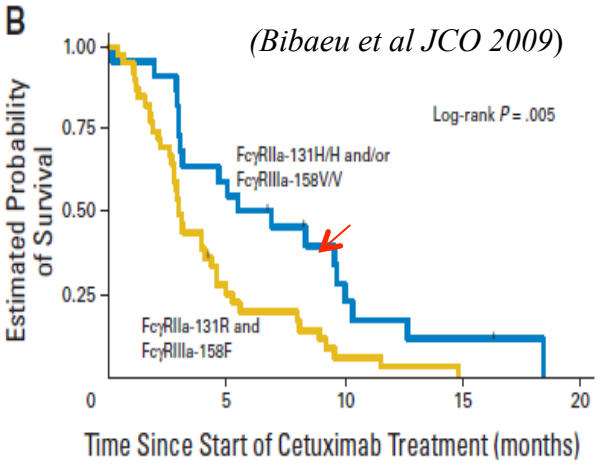
Adapted from: Cartron G, et al. Blood. 2002; 99(3):754-758.



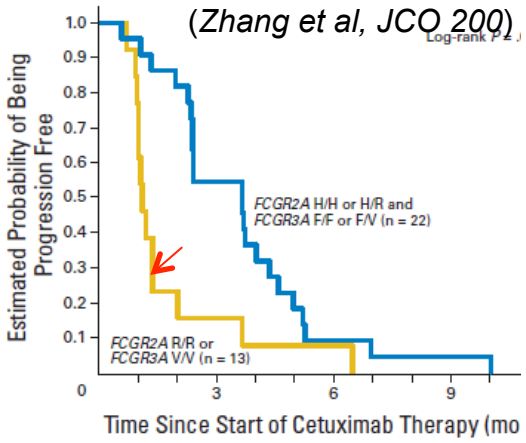
Adapted from: Weng W-K and Levy R. Journal of Clinical Oncology. 2003; 21(21):3940-3947.

Impact of in FcγRIIIa in mAb Activity

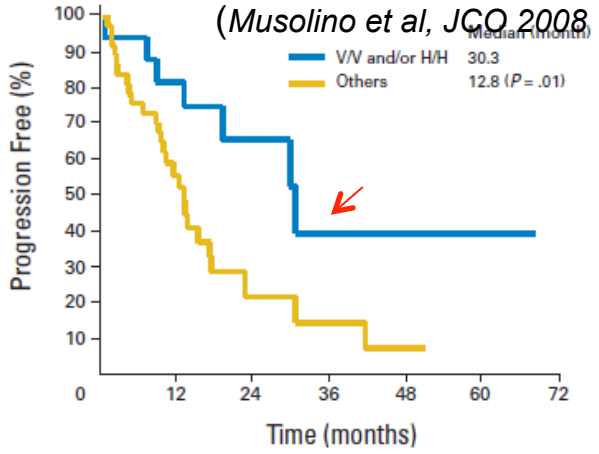
- **Conflicting results from other studies:**
  - **FcγRIIIA 158 V/V <u>not</u> predictive for rituximab in**
    - **CLL, or**
    - **rituximab + chemo in NHL**
  - **<u>In solid tumors</u>: results inconsistent**



C225 + CPT-11 (n=69) :
FcγRIIa-131H/H and <u>FcγIIIa-158V/V</u>: better PFS than 131R and 158F carriers



C225 alone (n=39):
FcγRIIa-131H and <u>Fc γIIIa-158F</u> better than 131R and 158 V



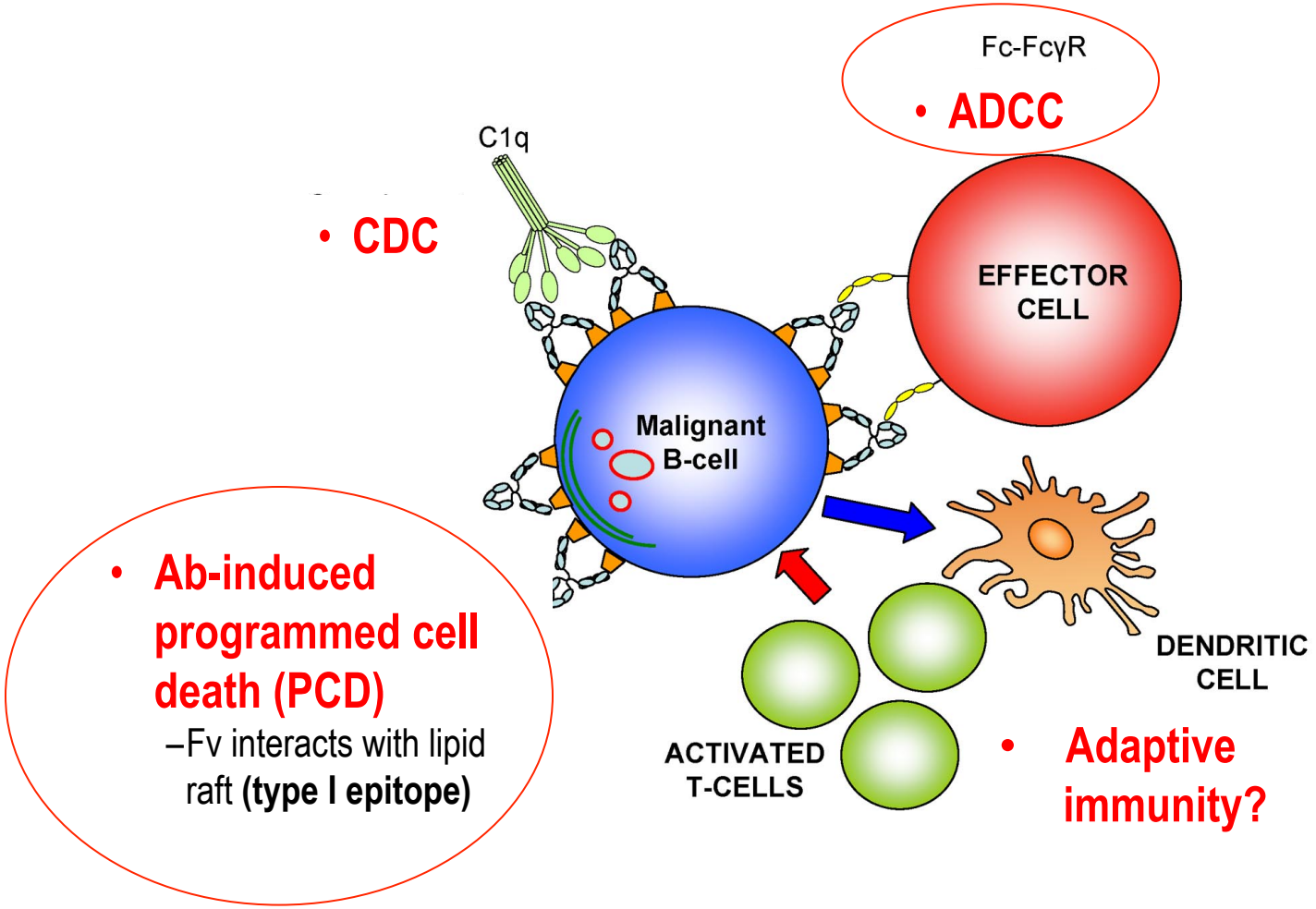
Trastuzumab +taxol (n= 54):
FcγRIIa-131H and <u>Fc γIIIa-158V/V better</u>

Factors that may impact the Fc-mediated innate host immunity

- **Host factors**:
  - FcR polymorphism
  - Type of effector cells (PMN, NK, macrophages) and FcRs involved in the interaction
- **Tumor factors:**
  - Tumor microenvironment may be suppressive of NK and CTL
  - **Access to effector cells**
    - Solid vs. liquid tumors
    - Bulky vs. minimal residual diseases
- **mAb factors …**
  - **Fv:** affinity and epitopes
  - **IgG1** vs. IgG2 … *IgG1 if ADCC is desirable. IgG2 to avoid ADCC (e.g. for host-cell targeting mAbs)*
  - **Fc chemistry**
    - A.A. sequence
    - Glycosylation (fucose content)

Improving the features of mAb - Example of anti-CD20 mAbs

Rituximab: a prototype anti-CD20 mAb (chimeric IgG1)



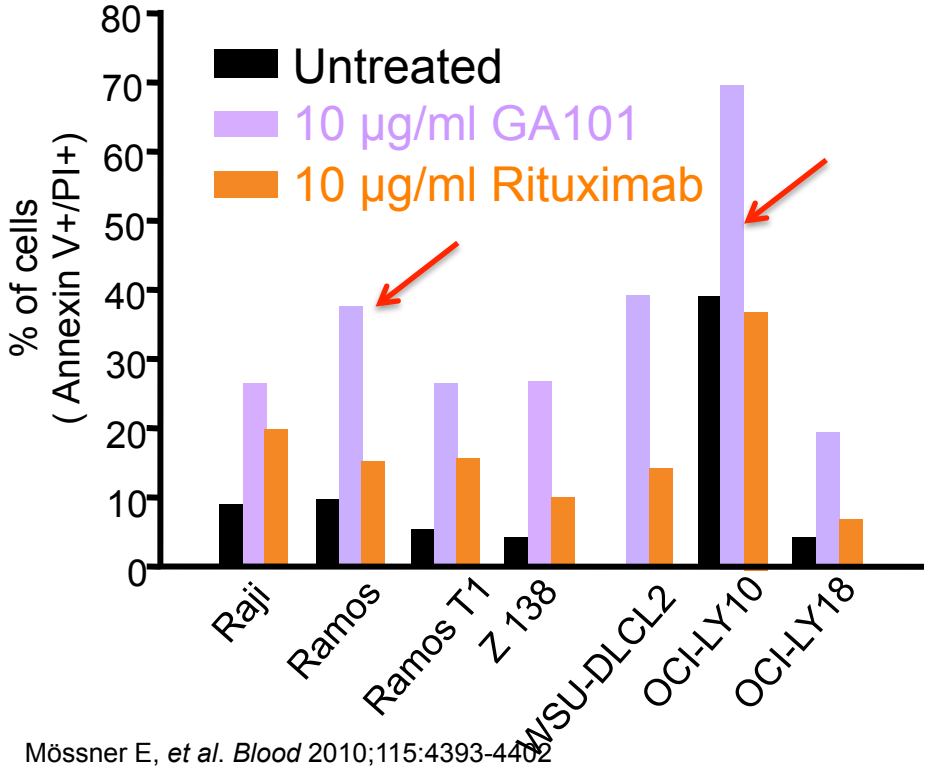
Evolution of anti-CD20 mAbs

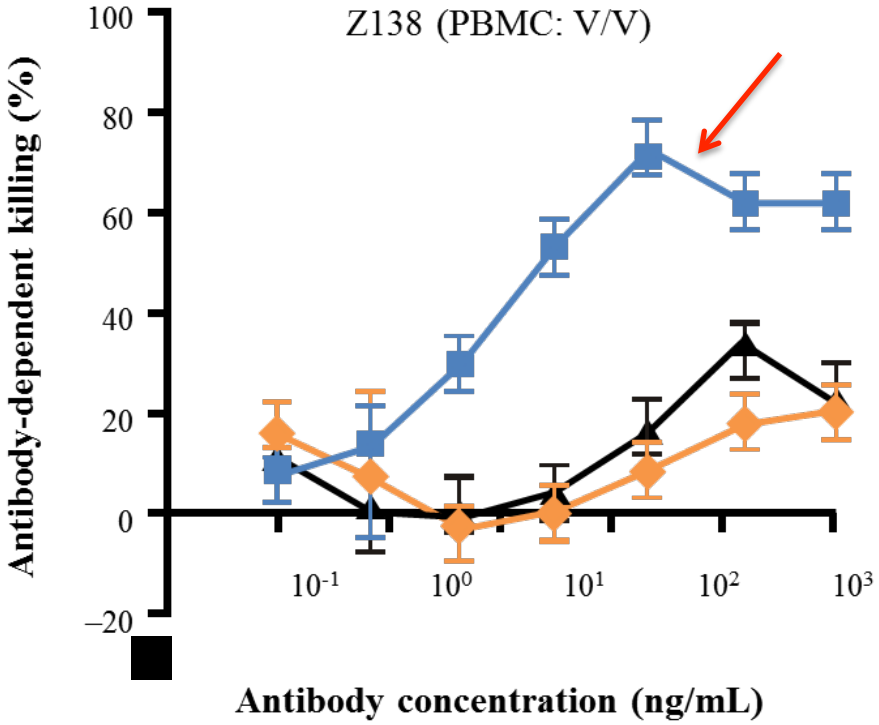
- **2nd generation … humanized mAb**
  - **Ofatumumab** – c/w rituximab:
    - **Type I epitope** (closer to membrane, slower off rate) → ↑ **CDC (10x)**
    - **Clinical** activity:
      - CLL: ORR in refractory CLL (58%, 47%) – FDA approved;
      - FL: 11% in rituximab-refractory tumors
- **3rd generation …Fc modification**
  - **AME-133v**
    - **Type I epitope**, higher affinity
    - **Fc modified (a.a. substitution)** → ↑affinity for 158 F/F; ↑ADCC (5-7X)
    - **Phase I – ORR 5/23 in FL in pts with low-affinity FcR (158 F/F or F/V)**
  - ***GA101***
    - **Type II epitope** → more Programmed Cell Death
    - **Fc modified (non-fucosylated)**

1. FDA approval; 2. Forero-Torreset al, CCR 2012; 3. Sehn et al, ASH 2011

GA101 Demonstrated Increased Direct Cell Death (DCD) and ADCC

- **More direct cell death with GA101 vs rituximab**
- **~ 100-fold higher ADCC than rituximab and ofatumumab**





Mössner E, *et al*. *Blood* 2010;115:4393-4402

Randomized Phase 2 Trial in Relapsed iNHL

(GAUSS Trial)

- GA101 vs. rituximab in patients had prior rituximab more than 6 months before study (N = 175)

Response at End of Induction (Primary Endpoint)

Patients with follicular lymphoma	Rituximab (n = 75)	GA101 (n = 74)
Overall response rate (ORR)	20 (26.7%)	**33 (44.6%)**
CR/CRu	3 (4.0%)	**4 (5.4%)**
PR	17 (22.7%)	**29 (39.2%)**

Sehn L et al. Oral presentation. *Blood*. 2011;118 (abstract 269).

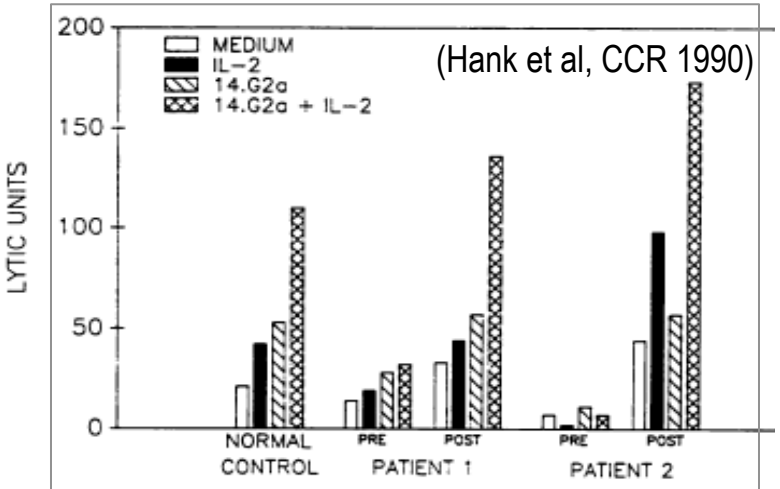
- **Clinical data with Fc-modified anti-CD20 mAbs were interesting *but*,**
  - Contribution of ADCC effects uncertain
    - both Fv vs. Fc were modified from rituximab
  - No Head to head comparison to rituximab in rituximab naïve patient
- **Other Fc-modulated mAbs in development**: CD19, HER2 …

Combination of mAbs with cytokines

- *Example of chimeric anti-GD2 mAbs (Ch 14.18) in neuroblastoma*

Background:

- **GD2:** overexpressed in neuroblastoma, melanoma
- **Chimeric anti-GD2 (ch14.18)** produced at NCI in 1989
- **In vitro findings**: ADCC by anti-GD2 mAb was by GM-CSF or IL-2



ADCC GD2 mAb or IL2 or combination

Early clinical experience with ch14.18

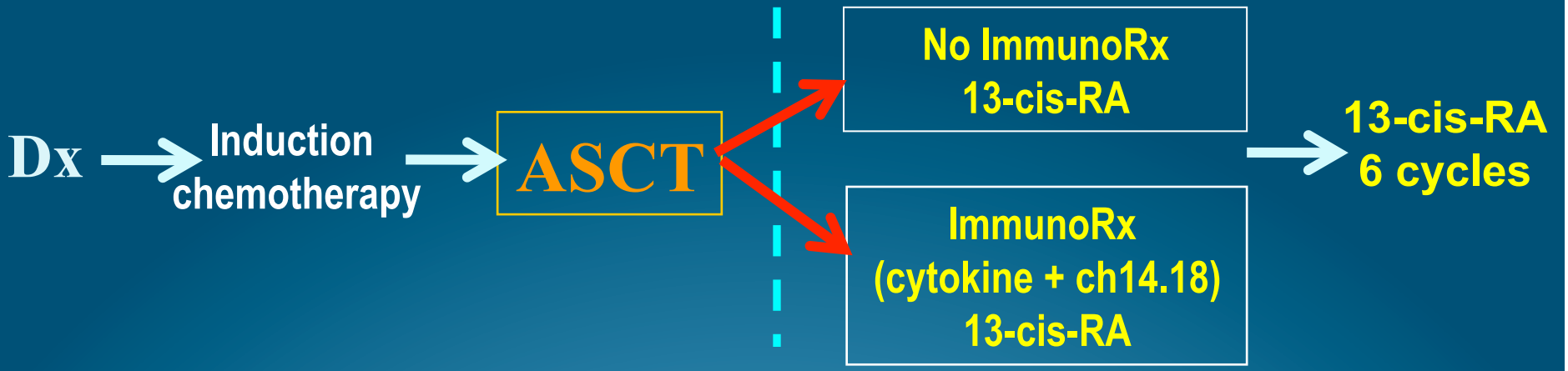
- Single agent in advanced disease → modest activity (<10%)
- Combination with GM-CSF → encouraging activity (20-30%)
- Pilot study of ch14.18 +GM-CSF/IL2 in MRD (CCG0935) → feasible

Hypothesis: Ch14.18 plus cytokines may be active in NB MRD

- **2001: Phase III trial ANBL0032**

PI: Alice Yu
Children's Oncology Group
Sponsor: CTEP

ANBL0032 – a phase III trial with immunotherapy + Ch14.18 in high risk neuroblastoma



Experimental arm: immunotherapy

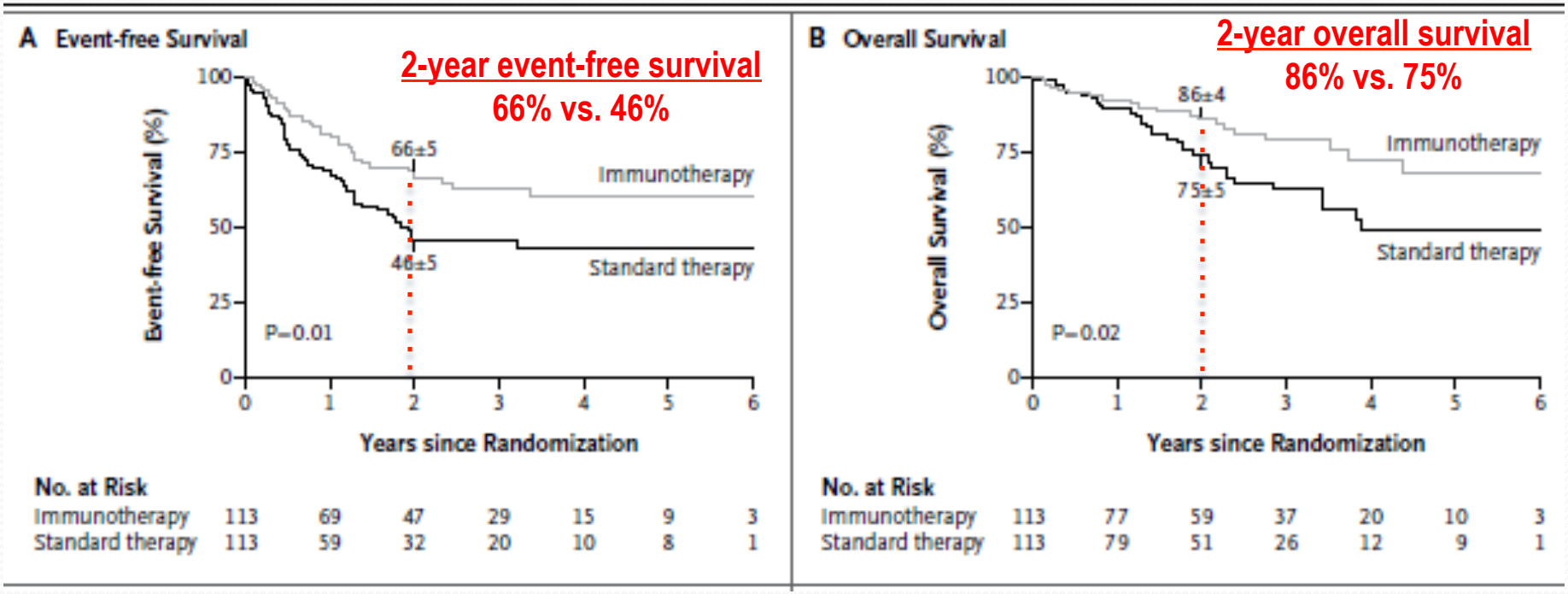
Schema for the administration of 5 courses of ch14.18 and cytokines

Course 1	Course 2	Course 3	Course 4	Course 5
Ch14.18	Ch14.18	Ch14.18	Ch14.18	Ch14.18
GM-CSF	Aldesleukin (IL-2)	GM-CSF	Aldesleukin (IL-2)	GM-CSF
RA	RA	RA	RA	RA

PI: Alice Yu
Children's Oncology Group
Sponsor: CTEP

Ch14.18 + Cytokines Improves Event-free Survival and Overall Survival For High Risk Neuroblastoma

Yu et al, NEJM 2010



Additional development of anti-GD2 mAbs:

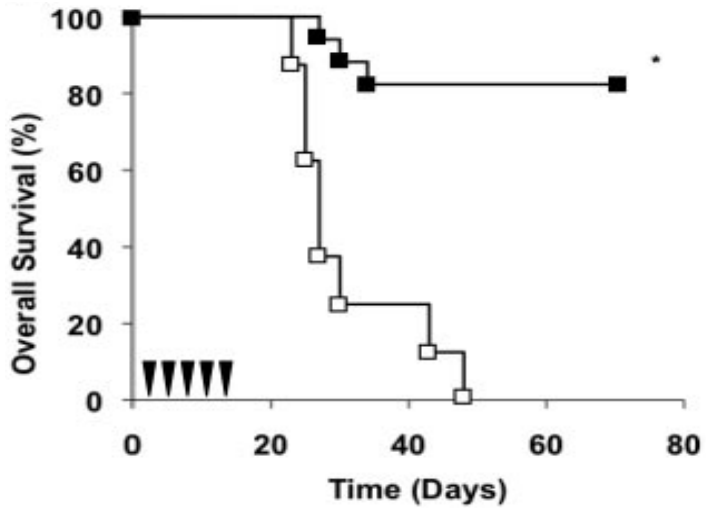
- Combination with lenalidomide to enhance effector function
- Ch14.18-IL-2 fusion protein
- Humanized version

Can passive immunotherapy (e.g. Tumor-targeting IgG mAb) induce active immunity?

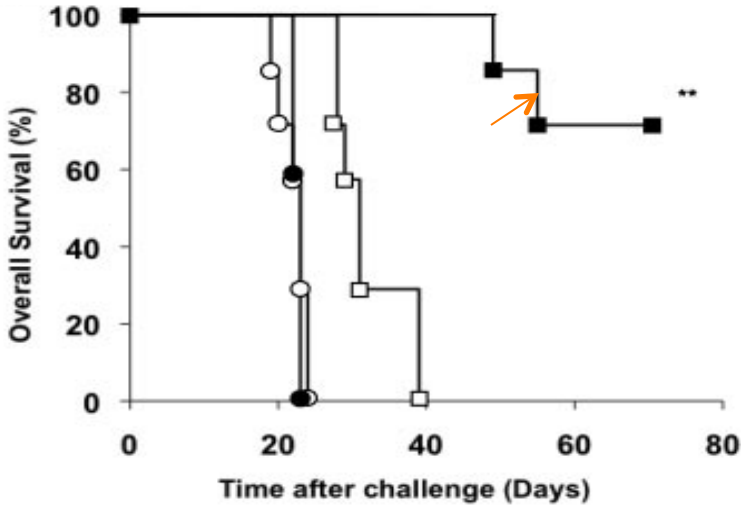
Can passive immunotherapy induce active immunity?

- ***Preclinical data***: anti-CD20 mAb protected mice from tumor challenges *(Abes et al, Blood 20010)*

First Rx with rituximab x 2wks



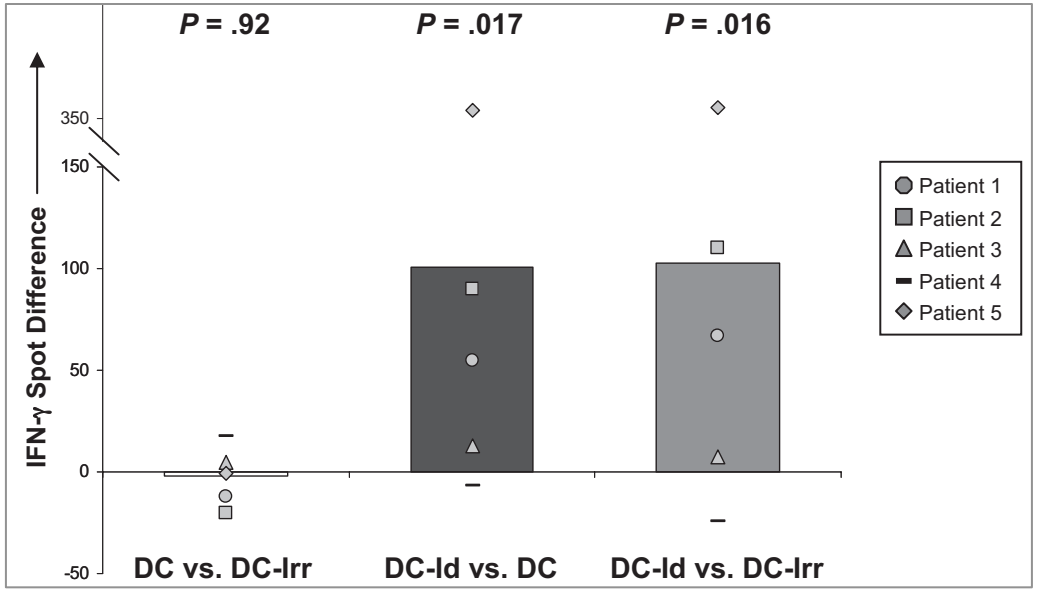
Rechallenge with tumor 70 days later



- Protection not transferrable via sera. Required CD4 /CD8 cells
- Protection was specific to CD20+ tumors
- Protection cannot be achieved by other cytotoxic agents (indicating possible requirement of Fc/FcR interaction rather than just Ag release from cell kill)

Can passive immunotherapy induce active immunity?

- ***In patients,*** Rituximab induced lymphoma idiotype-specific T cell response *(Hichey et al, Blood 2009)*



ELISpot assay for IFNγ-producing T-cells:

- Id-specific T cells significantly increased after rituximab therapy

- **Rituximab capable of inducing active immune responses**
- **No evidence that this mechanism is necessary or sufficient for efficacy**
- **However, such a potential mode of action is attractive and should be explored for optimization**

National Cancer Institute

OUTLINE

- Basic concepts
  - Immunology of Ab
  - Types of Ab-based therapies
- Unmodified full IgG mAb
  - Mechanisms of action through Fv and Fc;
  - Approaches to optimization
- ***Novel constructs to expand the "effectors"***
  - Redirecting T- cells to cancer cells
  - Redirect drug payloads to cancer (Ab-drug conjugates)
  - … *others*

Ab-Drug Conjugates (ADCs)

» Many ADCs

» Few successes

» Lessons learned

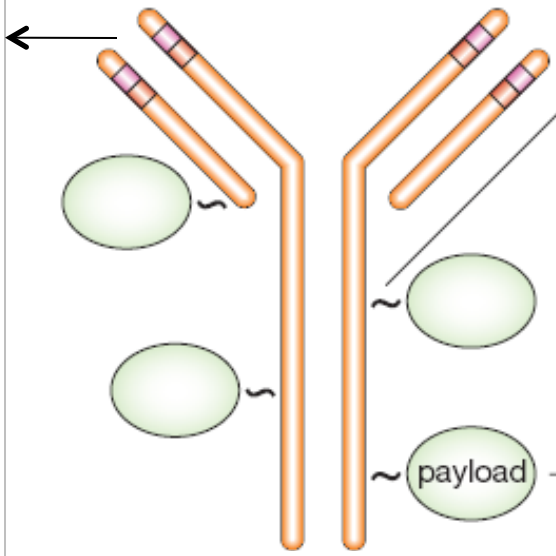
Ab-drug Conjugate (ADC) and Critical elements

Antigen targets:

Solid tumor:

- PSMA
- MUC1
- Mesothelin
- CanAg
- GPNMB
- CEA
- Integrin
- Cripto-1
- EphA2
- **Her-2**

Hem: CD19, 56, 70, **22, CD30**, 33



Linkers:

- **Hydrazone**– relatively unstable in plasma → premature release of chemo
- **Disulfide**
- **Thioether**
- **Peptide**
- …

  - Stable in plasma
  - Released chemo-linker in cells
  - reducible or non reducible → bystander killing of neighboring cells

Cytotoxic payload:

- Calicheamicin
- Maytansine (DM1/4)
- Auristatin (MMAE)
- Toxins

- Elements critical to success:
  - Target Ag expression in tumors vs. normal tissues
  - Linker selection
  - Intrinsic sensitivity of tumor cells to the chemotherapy

Activities of ADC

	Target	Activity
SGN-35 (Brentuximab vedotin)	CD30 (ADC)	• **ALCL**: 86% (57% CR) • **HD**: 75% (34% CR) **ORR was 10-20% with unmodified anti-CD30 mAb*
Trastuzumab-DM1 (T-DM1)	HER2 (ADC)	**Pts with HER2+ (IHC 3+ or FISH+) breast ca** • ORR **37.5%** *(Burris et al, JCO 2011)* **Phase III for T-DM1 vs. lapatinib + capcitabine** • Significant improvement in PFS and OS

Many ADCs had been in development ….

- Similar linkers and payload
- Similar to Her2 and CD30, targets are shared by normal tissues … ***Unlike CD30, normal tissues with solid tumor targets may be more prone to toxicities*** *(Skin, GI)*
- Target expression variable among patients …. ***Unlike T-DM1, reliable assays not always available to select tumors with overexpression***

Proper target and/or patient selection are essential to success with ADC

Re-directing T cells through mAb engineering

Examples:

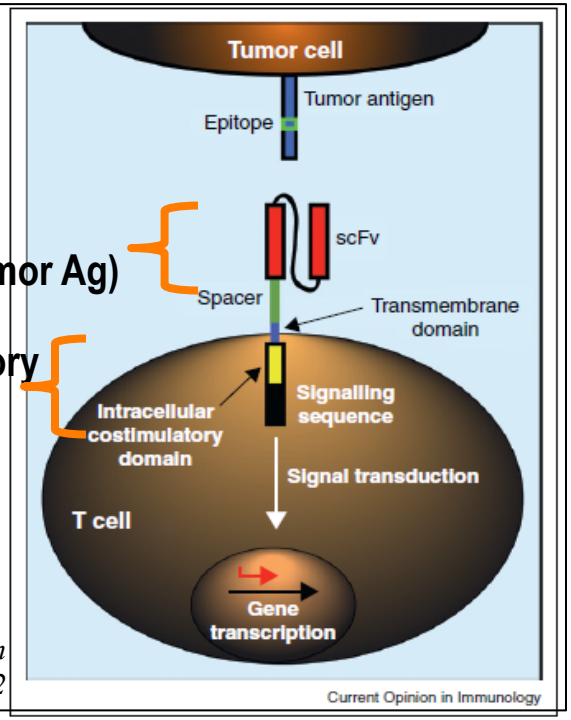
- T-body (Chimeric Ag Receptor, or CAR)
- Bispecific Mab - *Tumor Ag binding + CD3 binding*

Chimeric Ag Receptor (CAR)
– Replacing the TCR variable region with scFv

Engineered TCR for CAR → T-cell transduction

scFv (to tumor Ag)

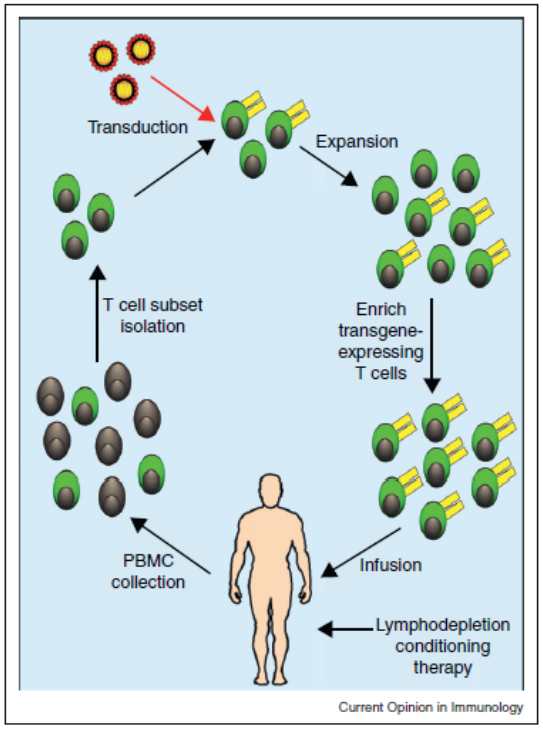
T-cell costimulatory molecule (CD28, CD3ζ or CD137)



Turtle et al, Cur Opinion in Immunol 2012

- 1st generation: scFv + TCR signaling domain
- 2nd generation: scFV + CD28
- 3rd generation: scFv + CD3ζ + CD28 or CD137 (4-1BB)

Adoptive transfer of CAR modified T cells



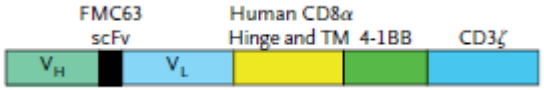
➢ ***Combing the diversity of mAb with potency of T cells***

Clinical trials to date

- FBP (folic acid R); CEA (GI); CAIX (RCC)…
- EGFRvIII
- **CD19; CD20**
- **HER2**
- ***others***

Clinical Experience: CD19 CAR

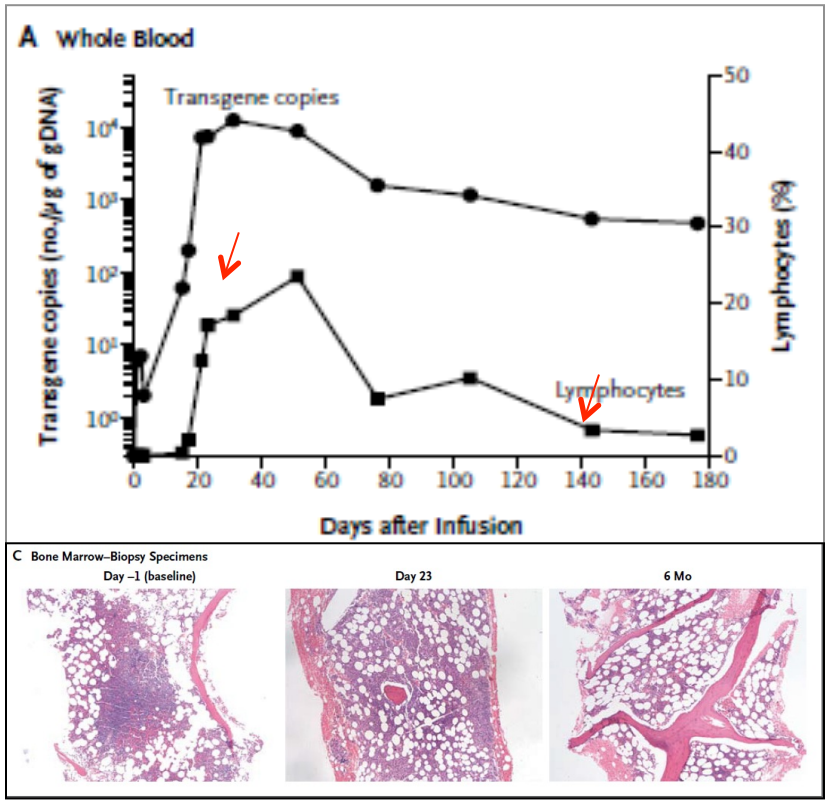
(Porter et al, NEJM 2011)



- ***Construct***: Anti-CD19 scFv + CD3-CD137 modified T cells
- ***Rx:*** nonablative myelosuppression → adoptive T cell transfer (1 x10^5 CD19 CAR cells)
- ***Patient***: w/ refractory CLL, received low dose

***Outcome*: Significant CD19-CAR T cell expansion around D10; Persistent after 6 months**

- ➢ **Cytokine release** (IFN-γ, CXCL9/10, IL6)
- ➢ **Tumor lysis syndrome**
- ➢ **Complete remission by D28**



(Kochenderfer et al, Blood 2011)

- Similar results from NCI using (scFv-CD3-CD28):

➢ **5 PR, 1CR (7-15+m) in 8 pts**

Clinical Experience: HER2 CAR

Case report : ***(Morgan et al, Mol Therapeutics 2010)***

HER2-CAR (ERB2 scFv + CD3-CD28-137) – modified T cell transfer in a patient with HER2+ colon cancer with lung and liver metastases

- Respiratory distress, cytokine storm 15 minutes after infusion. Died from multi-organ failure in 5 days
- **Autopsy**:
  - Lung alveolar damage; microangiopathy
  - CAR cell infiltrates mainly in LN and lungs
    - No differential distribution to tumor metastases (HER2 3+)
  - Low level of normal tissue (including lung) expression of HER2

- **The construct is highly specific and potent in activating T cells upon Ag recognition**
- **Low level of target expression in the lung appeared to make lungs the "first-pass" organ after HER2 CAR**

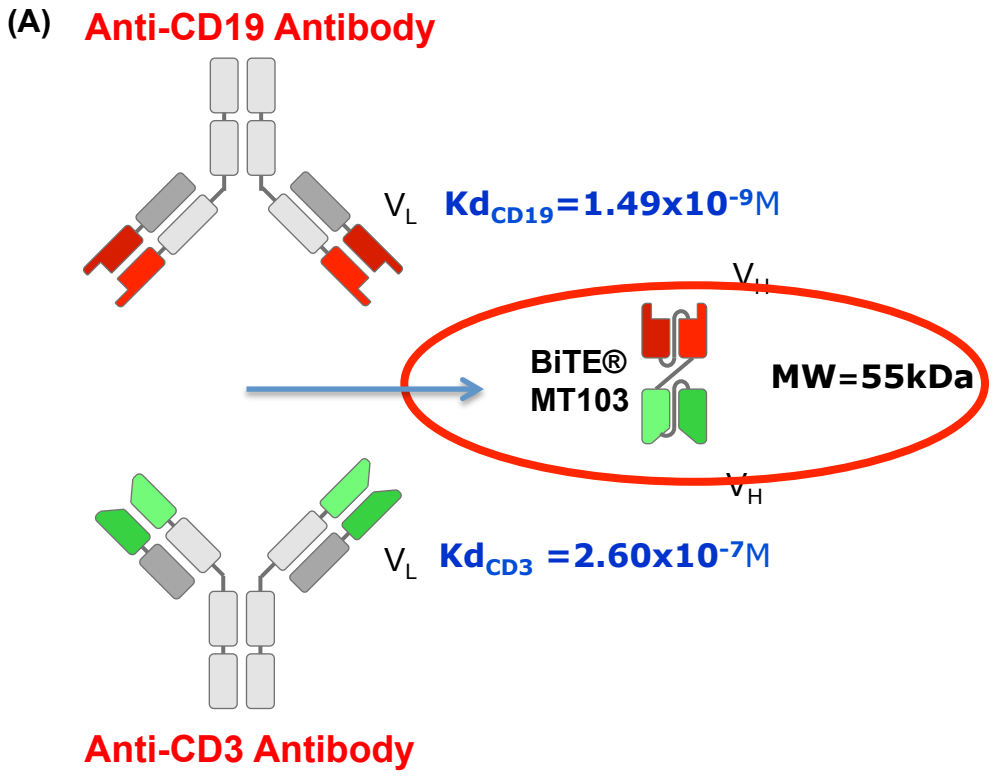
Re-directing T cells by Ab specificity

» **T-body (Chimeric Ag Receptor, or CAR)**

» **Bispecific Mab -** ***Tumor Ag binding + CD3 binding***

- Many attempts
- EPCAM x CD3 x (FcR) – *Trifunctional *Catumaxomab* – approved for malignant ascites for patients with ovarian cancer
- **CD19 x CD3 (CD19 BiTE) - **Blinatumomab***

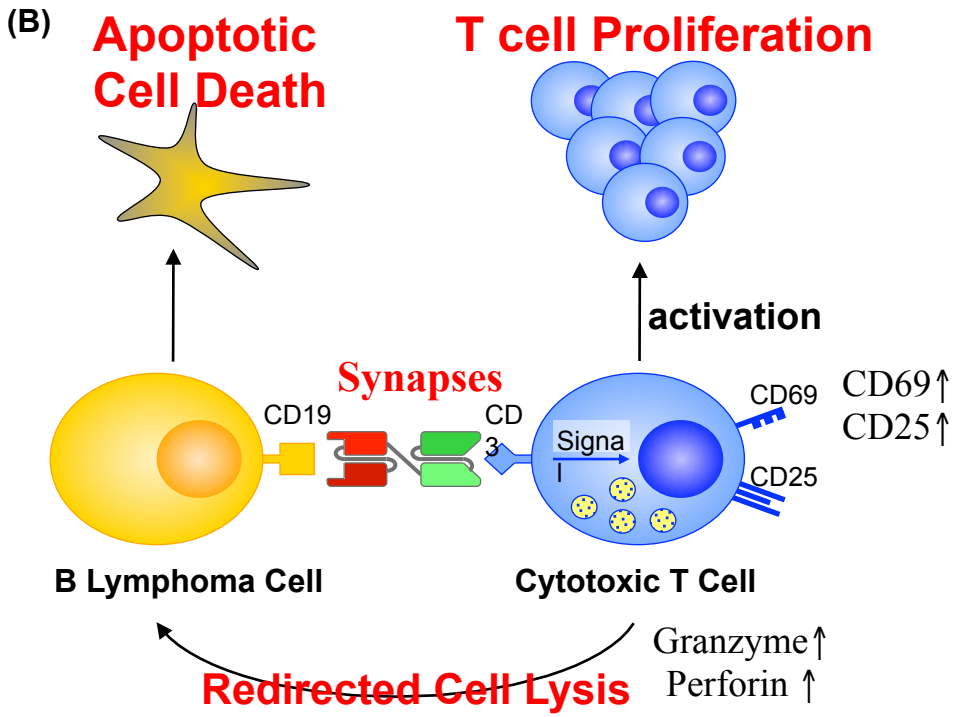
Blinatumomab (MT103), a Bispecific T-Cell Engaging (BiTE®) Antibody



Construct (Tandem scFv):

- Linking the VH-VL of a murine anti-19 mAb with an anti-CD3 mAb
- MW (55kD) – short half-life (2-3 hrs)

Blinatumomab (AMG-103) - MOA



Formation of cytotoxic T-cell synapses with tumor cell

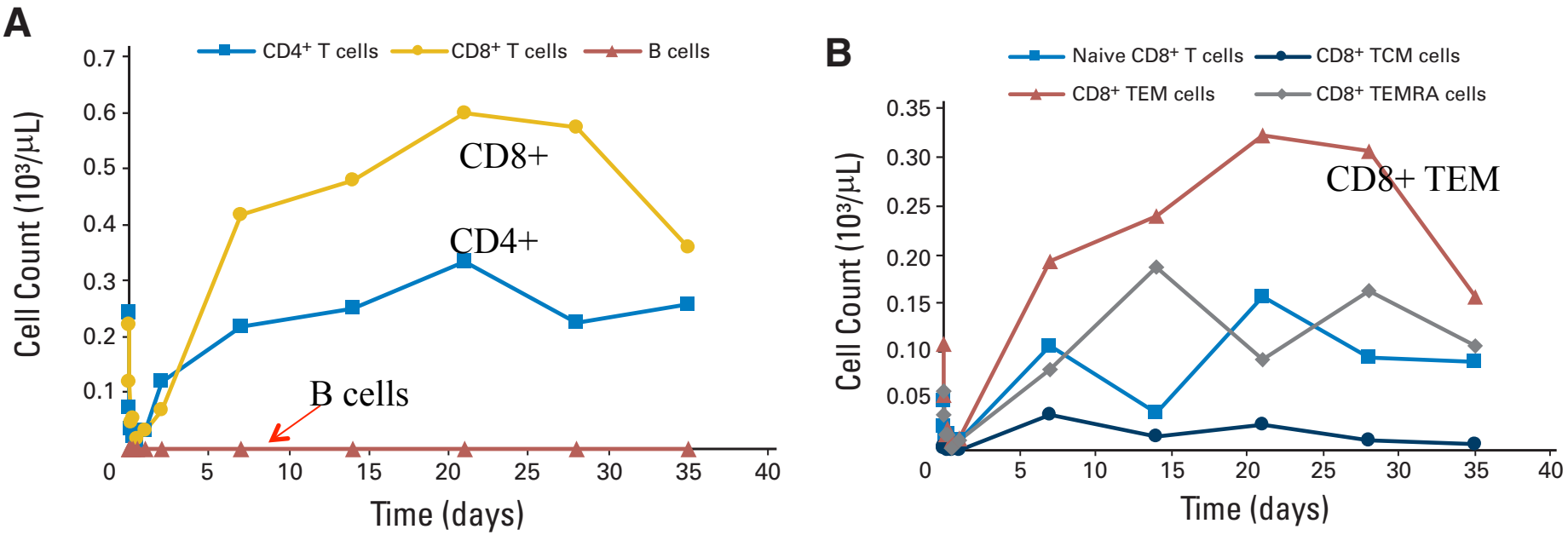
→ tumor and B cell lysis

→ T-cell proliferation and activation in situ

- **MHC independent, polyclonal activation of T cells, but only upon presence of target Ag**
- **Bypass typical T-cell immune suppressive mechanisms**
- **Does not require costimulatory molecules**

CD19 BiTE induced T-cell proliferation and maturation in patients

PBL from patients with ALL MRD treated with MT103



Effector memory T cell (TEM)
Central memory T cells (TCM)

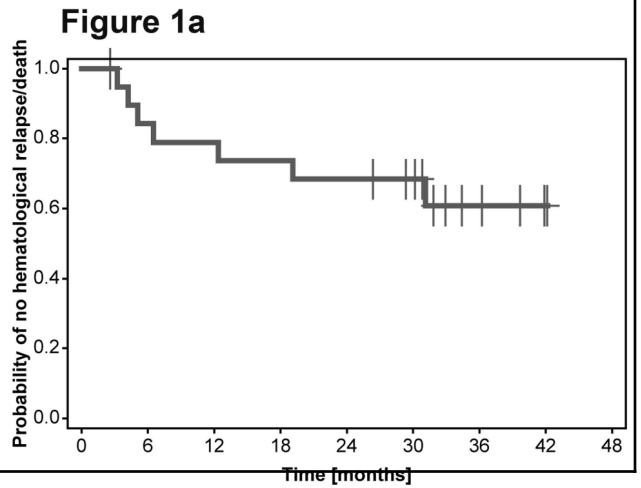
Topp et al JCO 211

CD19 BiTE activity in B-cell ALL

- **MRD:** Phase 2 in adult with MRD after chemotherapy *(Topp et al JCO 2011; Topp et al, Blood 2012)*

  15 μg/m²/d continuous infusion - 4 wks on /2wk off (*3 cycles after CR)

Molecular CR	Relapse free survival
16/20 (80%)	61% at 33 m • 9 pts had allo-HSCT: 65% RFS • 11 without HSCT: ~60% RFS



- **Relapsed/refractory diseases:** Phase 2 in adult ALL (*Topp et al ASCO 2012*

  Step-up dosing schedule 5→ 15 μg/m²/d

CR		Duration of CR
17/23 (72%)	44% CR 28% CRh	8.9 m (median)
*15/17 with molecular CR		

Pivotal trial in pediatric ALL MRD ongoing

CD19 BiTE in B-cell lymphoma

Phase 1 dose-escalation trial in B-cell NHL

- **Activity (dose dependent):**

2009 ASH Annual Meeting, abstract no. 2723

Dose Level	Patients	CR	PR	Overall RR
0.5 – 5 µg/m²/24 h	13	0	0	0/13
15 & 30 µg/m²/24 h	20	2	2	4/20
60 µg/m²/24 h	**9**	**3**	**5**	**8/9***
90 µg/m²/24 h	4	1	1	2/4#

*Durable PR/CR in MCL, CLL and FL

AEs:

- CNS events at 60 µg/m²/d: confusion, Seizure
  - Patients with low peripheral B cells at higher risk

* ***Mitigation strategy***: Stepwise increment to target dose (5 → 15 → 60 µg/m²/d)

Safety

- **Common toxicities with MT103**
  - Cytokine release syndrome – more serious in ALL with high tumor bulk
  - lymphopenia

- **Neurological /psychiatric AEs –**
  - G1-3: HA, dizziness, tremor, aphasia, encephalopathy, cerebellar syndrome, Seizure
    - Mostly occurring in cycle 1; reversible
  - Dose-related
    - *In ALL* (15 µg/m²/d): 2/20 discontinued therapy
    - *In NHL* (60 µg/m²/d or higher): 12 patients discontinued therapy
  - **Possible risk factor: Low peripheral B cell count at baseline**

Step-up dosing schedule and steroids feasible and effective in ALL

EpCAM BiTE (MT110)

The target:

- Epithelial adhesion molecule. Also present also on cancer stem cells.
- In tight junction in normal tissues

Phase I dose escalation trial in advanced solid tumors

(Fiedler et al, ASCO 2012)

- **Safety and Doses:**
  - **Not tolerable at > 10** µg/m²/d with standard schedule
    - **DLT: diarrhea, abdominal pain, LFT (*LFT associated with first dose)**
  - 5 different dosing schedules were explored
    - ➢ *Step up dosing required*

- **Activity** (n=43 evaluable, at different dose/schedules**)**
  - Reduction in circulating tumor cells. SD 35%
  - NO PRs

BiTE antibodies in development

•CD19	•EpCAM	•EGFR
•CD33	•CEA	•Eph2
		•HER2

What have we learned about T-cell engaging Ab- approaches

Bispecific antibodies, BiTE, CARs

- **Use of Ab to redirect T cells to target cells is a powerful strategy**
  - Potent and specific
  - MHC independent
  - May bypass typical immune-suppressive mechanism
- **Encouraging data in hematological malignancies**

Challenges:

- **T-cell activation can induce significant toxicities** (target-triggered cytokine release or target-mediated tissue damage)
  - *Challenging for many solid tumor targets*
- **Careful selection of target and development of mitigation strategy will be critical to achieving therapeutic window**

Summary and future directions

Summary

- **Exquisite specificity, and ability to carry "effector arms" (native or engineered) is unique among drug modalities**
- **Successes with: IgG mAbs targeting the tumor antigens, host immune cells and stromal factors; ADCs; Bispecific Abs**
- **New technology will continue to generate new designs and constructs**

Considerations:

- **Better understanding of MOA, especially in relationship to host immune system**
- **Identification / prioritization of targets**
  - cancer genome project (surface molecule with somatic mutations?)
  - Phase display library screen
- **… chose the right construct ("effector arm") appropriate for the target**
- **… in the right patients using biomarkers for patient selections**