

# Considerations for Designing New Trials with Chimeric Antigen Receptors: RAC Safety Symposium June 15, 2010

December 7, 2010



# Overview

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- ▶ **What are Chimeric Antigen Receptors (CARs)**
- ▶ **Impetus for RAC conference**
- ▶ **Options for trial designs**
- ▶ **Points to consider for design of Clinical Trials**

# Chimeric Antigen Receptors

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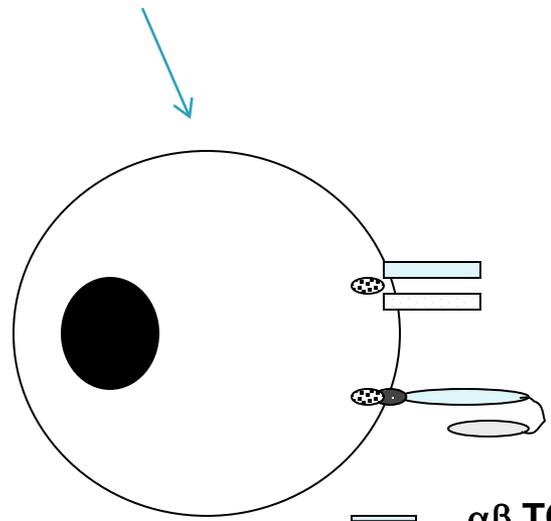
- ▶ A modified T cell receptor (TCR) that typically contains a immunoglobulin variable chain fragment (scFV) fused as a single chain to a TCR signaling domain with or without intracellular co-signaling motifs
- ▶ Offers recognition of surface tumor antigen by T cells
- ▶ Non-HLA restricted

# Chimeric Antigen Receptors

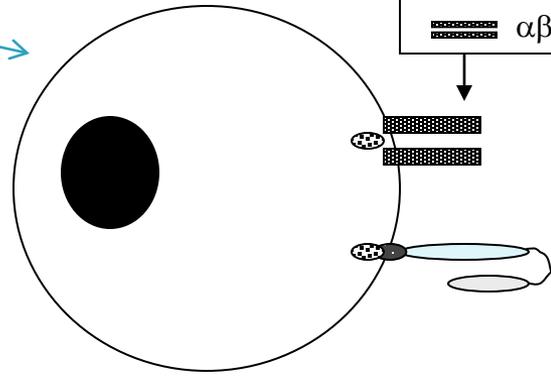
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- **1<sup>st</sup> Generation:** immunoglobulin signaling chain linked to the epsilon, gamma or zeta signaling sequences of the T cell receptor or the Fc $\gamma$ R.
- **2<sup>nd</sup> and 3<sup>rd</sup> Generation:** includes one (2<sup>nd</sup> Generation) or more (3<sup>rd</sup> Generation) co-signaling molecules, e.g. CD28, CD134, 4-1BB, to enhance survival and engraftment
- **Virus Specific:** Modification of virus-specific T cells, e.g. EBV-specific T cells with a CAR with or without co-signaling moieties

# 1<sup>st</sup> Generation



# Virus-Specific

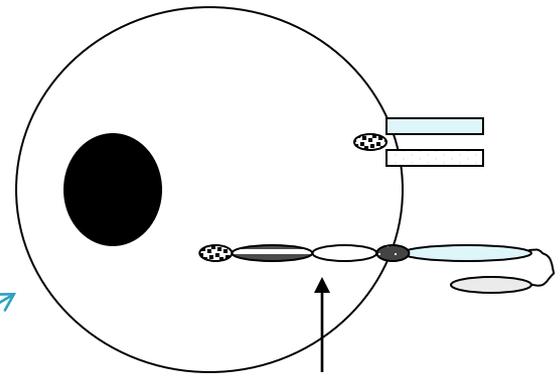


  $\alpha\beta$  TCR (EBV-Specific)

# CAR

-   $\alpha\beta$  TCR
-  TCR $\zeta$
-  Trans membrane domain
-  VH
-  VL

# 2<sup>nd</sup> and 3<sup>rd</sup> Generation



 CD28  
 4-1BB

# RAC Conference – June 15, 2010

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- ▶ Clinical trials using CAR T cells have shown some initial promising results, in particular a trial for neuroblastoma.\* However, two deaths on trials using CAR T cells, including one in which the death was assessed to be the result of an acute toxicity of the T cells prompted a review of the design of the trials with the goals of:
  - Enhancing the safe trial design for this new and promising therapeutic approach;
  - Informing the RAC and IBC review of future trials; and
  - Fostering exchange of information amongst leaders in the field.

\*Pule, *et al.* , *Nature Medicine*, 2008. 14(11):1264

# Trial Design Using CARs

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- ▶ **Initial studies used 1<sup>st</sup> generation CARs**
  - Limited persistence of cells
  - While some biological activity possibly indicative of clinical response, no efficacy shown with the initial antigens tested.
  
- ▶ **As limited persistence of cells was identified as a hurdle to achieving efficacy, strategies were developed to promote engraftment of CAR T cells.**

# Trial Design Using CARs

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- ▶ **Data from virus-specific CAR T cells showed enhanced persistence with co-activation of viral T-cell receptor.**
- ▶ **Trials using unmodified Tumor Infiltrating Lymphocytes showed increased persistence of cells and clinical efficacy upon:**
  - Lymphodepleting chemotherapy and/or radiation
  - Cytokine support for cells

# Strategies to Enhance Persistence and Engraftment of CAR T-Cells

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- ▶ **Use of Lymphodepleting Chemotherapy**
  - Data support that such chemotherapy creates “space” for T cells, alters certain chemokines and eliminates regulatory T cells
    - General consensus that CAR T cells, with the exception of virus-specific T cells, are unlikely to persist in the absence of preconditioning, however, there was some concern whether enhancing engraftment in early trials could enhance toxicity.
- ▶ **Use of Co-signaling Moieties**
  - Likely to make cells less resistant to activation-induced cell death but evaluations of which ones are optimal remains desirable

# Strategies to Enhance Persistence and Engraftment of CAR T-Cells (2)

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- ▶ **Cytokine Support, e.g. IL-2**
  - While cytokines should promote persistence, questions remain about when needed, optimum dose and potential to contribute to toxicity of cells

# Strategies to Enhance Persistence and Engraftment of CAR T-Cells

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- ▶ **Use of Virus-Specific CD8<sup>+</sup> Lymphocytes**
  - In published data for neuroblastoma, persistence and clinical effect was not dependent on lymphodepletion and cytokine support was not used.
- ▶ **The effects of age and prior stimulation (i.e viral exposure) on the potency/persistence of EBV or other virus-specific CAR T cells is unknown.**

# CAR T cells – Potential Toxicity

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- ▶ **The potential for CAR T cells to recognize low levels of antigen can potentially lead to toxicity**
  - Risk likely related to normal distribution of target antigen, dose, avidity and persistence of cells
- ▶ **Persistence of cells and engraftment may increase long-term toxicity and possibly acute toxicity**

# CAR T cells –Acute Toxicity

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- ▶ **Immediate binding to low levels of antigen on normal tissues can potentially lead to significant early toxicity through T cell activation and cytokine release**
- ▶ **Possible strategies to mitigate these risks:**
  - Conservative dose escalation
  - Split dose infusions over one or more days
  - Start without preconditioning chemotherapy
  - Start with 1<sup>st</sup> generation CARs
  - Include suicide genes

# CAR T cells – Acute Toxicity (2)

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- ▶ **Strategies to mitigate these risks raises other considerations:**
  - **Conservative dose escalation**
    - Ethical considerations in balancing safety in initial trials and desire to choose a dose with potential biological activity in patients with no other therapeutic options
  - **Split Doses over one or more days**
    - Need to define optimum monitoring parameters to detect subclinical signs of toxicity

# CAR T cells – Acute Toxicity (3)

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- **Include suicide genes**
  - Inclusion of some suicide genes could prompt immune reactions and/or require investigational drug (e.g. dimerizer to activate suicide gene)
  - General consensus that acute toxicities, which can occur within minutes, will unlikely respond to suicide genes
- **Start with 1<sup>st</sup> generation CARs with or without preconditioning chemotherapy**
  - Will the lack of toxicity translate into a similar profile for 2<sup>nd</sup> and 3<sup>rd</sup> generation CAR T cells?

# CAR T cells – Late Toxicity

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- ▶ **Persistence of CAR T cells may lead to long-term depletion of cells, which are important for normal human function, e.g. targeting CD19 will deplete normal B cells as well as tumor cells.**
  - Must consider the medical management of these long-term consequences
  - Suicide genes might be considered if medical management likely to be inadequate in the long-term

# Optimum Starting Doses

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- ▶ **Precise recommendations for starting doses across protocols are not possible**
- ▶ **Factors to be considered:**
  - **Preclinical models**
  - **Distribution of antigen on normal tissue, i.e. potential for on target – off tissue toxicity**
  - **Previous experience with CAR T cells**
  - **Use of 1<sup>st</sup> generation as opposed to 2<sup>nd</sup> or 3<sup>rd</sup> generation CAR T cells**
  - **Use of preconditioning chemotherapy may permit more rapid expansion of 2<sup>nd</sup> and 3<sup>rd</sup> generation and virus-specific T cells.**

# Optimum Starting Doses (2)

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- ▶ Comparing doses across trials is difficult as protocols use different approaches, i.e. cells/kg, cells/m<sup>2</sup> or total cells.
- ▶ In general, 2<sup>nd</sup> or 3<sup>rd</sup> generation CAR T cells should start at lower doses than 1<sup>st</sup> generation. Virus-specific T cells may behave like 2<sup>nd</sup> or 3<sup>rd</sup> generation CAR T cells.
- ▶ If lymphodepleting chemotherapy is being used, a lower starting dose than in non-conditioned patients should be considered.

# General Points to Consider (1)

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- ▶ **A clear plan for monitoring should be in place and at a minimum include provision for collection of:**
  - Physiologic data.
  - Cytokines, e.g., IFN- $\gamma$ , IL-6, TNF- $\alpha$  and others.
  - Plasma and peripheral blood mononuclear cells (PBMCs) for cryopreservation.
  - Routine labs from sera and urine.
  - Target organ specific labs as indicated.
- ▶ **Subject screening for adequate pulmonary and cardiac function**
  - Early reporting of SAEs and clinical outcomes are encouraged.
- ▶ **Uniform dosing using a weight based approach, e.g. cells/kg, would facilitate comparison of trials.**
- ▶ **Protocols that use retroviral or lentiviral vectors should monitor for the possibility of insertional mutagenesis.**

# General Points to Consider (2)

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- ▶ **The informed consent should:**
  - Discuss the risk of insertional mutagenesis.
  - Employ the terms “gene transfer” and not “gene therapy” to avoid the potential for therapeutic misconception.
- ▶ **Special considerations requiring extra care:**
  - Effects of CAR expression on non-T cell populations, e.g. NK cells.
  - Effects of CAR expression on specific T cell subsets, e.g. CD8<sup>+</sup> T cells vs. all T cells or subsets of T cells, e.g., memory vs. effector T cells.
  - Novel vectors used to improve transduction efficiency.
  - New cytokine support regimens.
  - New preconditioning regimens.

# 1st Gen. CARS – Points to Consider

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## ▶ Governing Principles

- 1<sup>st</sup> generation CAR T cells have shown the ability to induce inflammatory side effects.
- The survival of 1<sup>st</sup> generation CAR T cells appears to be limited *in vivo* likely due to the lack of co-stimulatory moieties.
- Preconditioning will likely not enhance survival of 1<sup>st</sup> generation CAR T cells *in vivo* but is unlikely to significantly increase the potential for toxicity from the cells.

# 1st Gen. CARs: Points to Consider (2)

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- ▶ **Governing Principles, cont. . .**
  - **Cytokine support may enhance the transient proliferation and survival of transferred cells but, used in isolation, will likely not result in engraftment.**
  - **Trials may provide a preliminary indication of safety, but to date clinical benefit has not been seen with the antigens tested.**

# 1st Gen. CARs: Points to Consider (3)

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## Clinical Strategies

- ▶ Based on data from trials to date, starting doses of approximately\*  $3 \times 10^6$  to  $1 \times 10^7$  cells/kg have been tolerated, but have had limited clinical benefit.
- ▶ A dose limiting toxicity was seen in a European trial using a 1<sup>st</sup> generation CAR against carbonic anhydrase IX for renal cell cancer after repeated infusions of T cells at doses starting at approximately  $3 \times 10^5$  cells/kg on day 1 to  $3 \times 10^7$  cells/kg on days 3 and 5.
- ▶ Trials using CARs against novel targets may need to be started at a lower dose.

\* Dose conversions done using  $37 \text{ kg/m}^2$  or assuming 70 kg subject

# 1st Gen. CARs: Points to Consider (4)

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## Clinical Strategies

- ▶ **Preconditioning is unlikely to enhance persistence.**
- ▶ **Cytokine support should be considered on an individual basis relative to the goals of the study. Adequate doses of cytokines that support transient T cell proliferation and survival without undue toxicity may be considered.**

# Viral Specific 1<sup>st</sup> Gen. CAR T cells

## Points to Consider

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### Governing Principles

- ▶ The survival of EBV-specific T cells expressing 1<sup>st</sup> generation CAR has the potential to be enhanced by engagement of the endogenous TCR/ costimulatory pathways on non-tumor cells, e.g. infected B or epithelial cells.
- ▶ Preconditioning may not be needed to enhance the engraftment of EBV-specific T cells expressing 1<sup>st</sup> generation CARs.
- ▶ Proliferation of EBV-specific T cells expressing 1<sup>st</sup> generation CARs *in vivo* may be triggered by endogenous viral antigens at doses as low as  $2 \times 10^7$  cells/m<sup>2</sup> (approximately  $5 \times 10^5$  cells/kg). This dose has been associated with therapeutic effects.

# Viral Specific CAR T cell: Points to Consider (2)

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## Clinical Strategies

- ▶ Based on data from trials to date, moderate initial cell doses of approximately  $5 \times 10^5$  cells/kg ( $\sim 2 \times 10^7$  cells/m<sup>2</sup>) have been well tolerated.\*
- ▶ Initial doses for CAR T cells targeting novel antigens may need to be lower.
- ▶ Preconditioning may not be necessary to enhance persistence.
- ▶ Additional studies should be considered to assess if cytokine supplementation improves clinical outcome.

\* Based on lack of reports of SAE or deaths attributed to the T cells.

# 2<sup>nd</sup> and 3<sup>rd</sup> Gen. CAR T cells: Points to Consider

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## Governing Principles

- ▶ 2<sup>nd</sup> and 3<sup>rd</sup> generation CAR T cells have the potential for proliferation and long-term engraftment.
- ▶ Survival and engraftment are likely enhanced by preconditioning.
- ▶ Cytokine support of transferred cells may enhance initial cell proliferation and survival and should be investigated in more depth.
- ▶ Initial toxicity might be avoided by reducing cell dose and/or splitting doses.

# 2<sup>nd</sup> and 3<sup>rd</sup> Generation CAR T cells

## Principles (2)

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### Clinical Strategies

- ▶ Dosing is based on transfer of unselected CAR T cells; selection for subsets such as CD8<sup>+</sup> cells only may impact potential toxicity/potency.
- ▶ Data from trials to date, the majority of which have used 2<sup>nd</sup> generation CAR T cells, indicate initial doses ranging from approximately  $5 \times 10^5$  cells/kg to up to  $10^7$  cells/kg have been tolerated.

# 2<sup>nd</sup> and 3<sup>rd</sup> Generation CAR T cells

## Principles (2)

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### Clinical Strategies

- ▶ A serious adverse event possibly related to the T cells has been seen in a trial using a 3<sup>rd</sup> generation CAR T cells at a dose of approximately  $2 \times 10^7$  cells/kg.
- ▶ An acute toxicity and death on a trial using a 3<sup>rd</sup> generation CAR T cells occurred at a dose of approximately  $2 \times 10^8$  cells/kg.
- ▶ There is not one optimum starting dose for novel targets; it must be justified by preclinical data, distribution of the target on non-tumor tissue, signaling moieties, T cell subsets and use of preconditioning, and cytokine support.

# 2<sup>nd</sup> and 3<sup>rd</sup> Generation CAR T cells

## Principles (3)

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### **Clinical Strategies, cont. . .**

- ▶ **Inclusion of co–signaling moieties as a strategy to enhance T cell persistence and possibly efficacy is often combined with preconditioning chemotherapy to enhance engraftment.**
- **Strategies to achieve optimum engraftment while minimizing the potential for acute and long–term toxicity should continue to be studied.**

# 2<sup>nd</sup> and 3<sup>rd</sup> Generation CAR T cells

## Principles (3)

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### **Clinical Strategies, cont. . .**

- ▶ **Potential benefits of cytokine support should be explored further including:**
  - **Whether and when to include cytokine support**
  - **Which cytokine to use**
  - **Optimum dose**

# Conclusions

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- ▶ **CAR T cells have shown therapeutic benefit in initial trials, however,**
  - **The ability to activate T cells against tumor antigens in an MHC–unrestricted manner not only offers a potentially potent tool for immunotherapy but also raises the risk for severe acute toxicity.**
  - **Preclinical models have limited ability to predict such toxicities.**

# Conclusions (2)

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- **Precise recommendations regarding the starting doses are not possible at this time and it is critical to justify the starting dose based on preclinical data, type of T cell, trial design and experience to date.**
- **Active research questions remain regarding optimum use of co-signaling moieties, virus-specific T cells, T cell subsets, preconditioning, cytokine support and suicide genes.**

# Conclusions (3)

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- ▶ **Standard treatments for subjects enrolling in these trials also carry risks.**
  - e.g. Hematopoietic stem cell transplant may have a mortality risk of 5–10%
- ▶ **While it may not be possible to eliminate risk, the goal is to minimize risk, especially in early trials where benefit may also be less likely.**