Tumor Antigen Specific mAb Immunotherapy and Combinations for Head and Neck Cancer

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Disclosures

“Heal with Steel”

Amgen: Clinical trial/research funding
Bristol-Myers Squibb: Advisory board, Clinical trial/research funding
VentiRx Pharmaceuticals: research funding
BONUS QUESTION:
(50 points)
What's the name of that thing that hangs down in the back of our throats?
Two distinct diseases comprise HNC
Escalating Chemoradiation Morbidity

Convergence of “targeted therapy” with “immunotherapy”
The promise of tumor-targeted biological agents

- Agents that do not directly attack DNA
- Directed against important biological/molecular targets – sparing the normal cells (bone marrow)
- Well-tolerated drugs that can be combined safely with cytotoxics
EGFR - Human SCCHN (+ in 80-100%)
Cetuximab + RT in Locally Advanced SCCHN: Locoregional Control

- Median (mos) comparison:
  - RT (n=213): 19
  - RT+C (n=211): 36
- Events comparison:
  - RT: 106
  - RT+C: 90
- Significance: $p = 0.02$

Bonner, NEJM, 2006
RTOG 1016: A Randomized Phase III Trial of Chemoradiotherapy With Cisplatinum or Cetuximab in P16+ Oropharynx Cancer

**ELIGIBILITY**
- Stage III, IVA, B
- Resectable
- P16+
- Oropharynx Cancer

**RANDOMIZE**
- IMRT 70Gy/35 fx
- Cetuximab 400/250 mg/m² qwk
- Cisplatin 100 mg/m²/q21d

**Stratify:** HPV, smoking, stage

**Cetuximab loading dose = 400 mg/m² on Day 1 of Cycle1 with induction**

**IMRT = intensity-modulated radiation therapy.**

[Link to ClinicalTrials.gov](https://ClinicalTrials.gov)
EGFR Inhibition – immune mechanism of action?

Tyrosine kinase inhibitor (erlotinib, gefitinib)

Antibody (cetuximab, panitumumab)

Signaling cascades

Gene activation
Cell cycle progression

MYC - FOS - JUN

Nucleus

Adaptor proteins

PLC - GRB2

P P

↓ Proliferation

↓ Survival

↓ Angiogenesis
Cetuximab
anti-EGFR monoclonal Ab

- IgG1 (chimeric mAb)
- High-affinity and prevents ligand binding to EGFR
- ↑ apoptosis ↓ angiogenesis
- Clinical anti-tumor activity in ≈20% (Bonner, Vermorken), not correlated with level of EGFR expression or gene copy number
Structure of a mAb

Variable Portion
F(ab): epitope specific
In chimeric mAb this portion is murine

Fc Portion
Constant portion
(changes depending on the isotype)

EGFR mAb therapy in SCCHN: inhibition of phosphorylation or immunotherapy?
Cetuximab blocks EGFR activation but does not kill HNC cells

Lysis/apoptosis

<table>
<thead>
<tr>
<th>cetuximab µg/ml</th>
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<td>0</td>
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pEGFR

EGFR

β-actin

Lopez-Albaitero 2007
Antibody dependent cell cytotoxicity (ADCC)

- Polymerized mAb complexes trigger responses in macrophages and NK cells through the FcγR receptors.
- There are several types of FcγR on effector cells, these variations influence the response against a mAb depending on its isotype.
Potential immune effect of mAb for cancer therapy – explanation for variability in responses

Cetuximab mediated ADCC correlates with FcγR genotype

A

- PBMC
- NK cell depleted

% lysis vs. E:T ratio

B

- cetuximab+3G8 mAb
- cetuximab

% lysis vs. FcγR genotype

C

p<0.0001

% lysis vs. FcγR genotype

Lopez-Albaitero *CII* 2009
Progression-free survival for patients with metastatic colorectal cancer (mCRC) according to the presence or absence of (A) KRAS mutation and to the (B) Fc(γ)R polymorphisms combination.

Is cetuximab activity immune mediated?

N=104 patients

Disease-Specific Survival by FCgamma RIIIa

Srivastava, Clin Can Res 2013

Results confirmed with RTOG 0522 trial
Cetuximab activated NK cells secrete cytokines and chemokines associated with recruitment of T cells and dendritic cells – bridging a network of antitumor lymphocyte activation?
Cetuximab coated EGFR

tumor cell

effector cell

Cytolysis

Internalization of Ab-coated EGFR and cross presentation to T cells

Dendritic cell

T cell stimulation and expansion

Cross-presentation of tumor antigens by DC

ADCC

Fc

F(ab)
EGFR-specific tetramer+ T cell frequencies are elevated in cetuximab treated HNC patients compared cetuximab naïve HNC patients

- Cetuximab treated (n=17)
- Cetuximab naïve (n=39)
- HLA-A2- (n=21)
- HIV tetramer (n=37)

\[ p < 0.001 \]

Athanassios Argiris, MD
Michael Gibson, MD
James Ohr, MD
Pedro Andrade, MD

Srivastava, *Clin Cancer Res*, 2013
Neoadjuvant Cetuximab Followed by Surgery/CRT and adjuvant Cetuximab (UPCI Protocol #08-013)

Endpoints:
Modulation of biomarkers, 2-yr DFS
Sample size N=33/40
PI - Ferris
Neoadjuvant Cetuximab
Followed by Surgery and adjuvant Cetuximab
(UPCI Protocol #08-013)

Endpoints:
Modulation of immune biomarkers, 2-yr PFS
ACCRUAL 33/40

James Mountz, MD, PhD
Exhausted CD8⁺ T cells express PD-1 and Tim-3 during chronic antigen stimulation
Blocking inhibitory receptors to reactivate exhausted T cells

New immunotherapeutic targets: CTLA-4 and PD-1 (Programmed death 1)

TIM-3, PD-1, and LAG-3 Expression by CD8⁺ TIL and PBL (HPV+ vs HPV-)
Checkpoint Receptors on HPV E7-Tetramer+ TIL (HPV+ Patient 11-6369)

* % in tetramer + Cells

HPV+ sample contains higher proportion of PD-1+/TIM-3+ cells on HPV E7-tetramer+ TIL than PBL
Targeting the PD-1/PD-L1 pathway

Blocking Anti-PD-1 mAb

Blocking Anti-PD-L1 mAb

Topalian, NEJM, 2012
Brahmer, NEJM, 2012

Ferris, RL, Cancer, 2012
CA209-141: Randomized Phase II/III Trial of Nivolumab vs Cetuximab in Recurrent or Metastatic Platinum-refractory HNSCC

Study Population:
Recurrent or metastatic HNSCC, on or within ~6 months of last dose of platinum-based therapy.

Screening:
$n = 225$

Randomization & Stratification:
$n = 180$
- Stratification by HPV status and prior cetuximab treatment
- 2:1 randomization to N or IC

Treatment Arm N - Nivolumab:
- Nivolumab 3 mg/kg Q2 wks

Treatment Arm IC - Therapy of Investigator’s Choice:
- Cetuximab 400 mg/m² IV once then 250 mg/m² weekly
- Methotrexate 40 mg/m² IV push weekly
- Paclitaxel 80 mg/m² IV weekly
- Docetaxel 30 mg/m² IV weekly
- Capecitabine 1000 mg/m² orally twice a day for Days 1 - 14 followed by a rest of 7 days every 21 days

Treatment until confirmed progression or study drug discontinuation for any other reason. Post treatment follow-up for safety, progression events and overall survival.

Co-Primary Endpoint: PFS/OS
Secondary Endpoint: ORR
Cetuximab

Immune Responses induced by cetuximab treatment

TGF-β, CD39, CTLA-4?
Cetuximab induces suppressor/regulatory T cells in treated HNSCC patients

* paired Wilcoxon Sign test
Cetuximab induces CTLA-4+ Treg cells in treated HNSCC patients
Treg suppression of cetuximab mediated antitumor activity is mediated by TGF-β and correlates with clinical response. 

\[ p = 0.015 \] (n = 20)

CD4+CD39+CD25+ Treg before cetuximab treatment:
- Green: > 6%
- Blue: < 6%
Phase Ib Trial of Concurrent Cetuximab/IMRT with Ipilimumab, Plus Biomarker Correlatives, in Locally Advanced, High Risk Oropharynx Cancer

**SCHEMA**

- Stage III-IVA OPSCC (HPV-HPV+ smokers, $\geq$N2b)
- p16 IHC
- Tumor/Blood collection

**Cetuximab/Radiotherapy Plus Ipilimumab**

- RT 66 Gy with 200 cGy daily fractions in 6.5 weeks
- Cetuximab weekly at 250 mg/m$^2$ during radiation*
- Ipilimumab 3, 10 mg/kg q21 days, starting week 4

*after loading dose of 400 mg/m$^2$ on cycle 1, day 1
Ipilimumab will be continued at indicated dose for additional 2 cycles.

Ferris and Bauman
Gated on CD4⁺ T cells

A

\[\text{CD25} \quad \text{FOXP3} \]

- Isotype
  - 7.78
  - 40.1
  - 15.6

- Ipilimumab
  - 7.36
  - 41.7

- NK cells + isotype
  - 11.5
  - 40.4

- NK cells + Ipilimumab
  - 14.5
  - 27.2

B

\[\% \text{FOXP3}^+ \text{TIL-Treg} \]

- TIL: + + + + +
- NK: - - + + +
- Ab: Ipi Ipi Iso Ipi

\[p=0.04\]

\text{n.s.}

C

\[\text{CD25}^+ \text{FOXP3}^+ \text{TIL-Effectector} \]

- TIL: + + + + +
- NK: - - + + +
- Ab: Ipi Ipi Iso Ipi

\text{n.s.}
Summary

- Cellular immunity (antitumor NK and T cells) are induced by cetuximab

- Suppressive mechanisms include regulatory T cells (Treg) and checkpoint receptors (CTLA-4, PD-1, etc)

- But what else is clinically available for immunotherapy of HNC? -> Toll Like Receptor Adjuvants
  - TLR3 – poly IC:LC – plans for clinical trial
  - Active8: EXTREME +/- TLR8 agonist trial
Human TLR Family and Known Ligands

- TLR 5: Flagellin, Lipopeptides
- TLR 1/2/6: LPS
- TLR 4: Bacteria

Virus (e.g., Polio, HIV, influenza):
- TLR 7: ssRNA
- TLR 8: dsRNA
- TLR 9: CpG DNA

Unknown:
- TLR 10: MyD88

Cell membrane, Endocytosis, Endosome, Nucleus
**The Nobel Prize in Physiology or Medicine 2011**

**1. Innate immunity**
Components of microorganisms bind to Toll-like receptors located on many cells in the body. This activates innate immunity, which leads to inflammation and to the destruction of invading microorganisms.

**2. Adaptive immunity**
Dendritic cells activate T lymphocytes, which initiates adaptive immunity. A cascade of immune reactions follows, with formation of antibodies and killer cells.
Toll-like Receptor 8 (TLR8) Pathway is Important in Human Immune Responses

- Activation induces potent Th1 immune response
- Expressed on myeloid dendritic cells (CD11c+), monocytes (CD14+), and natural killer cells (CD56+) in humans
- Induces significant IL-12 production in humans
- Can be activated by small molecule agonists
# TLR8 – expression and function

<table>
<thead>
<tr>
<th>Expression</th>
<th>Myeloid DC, Monocytes</th>
<th>Plasmacytoid DC</th>
<th>Plasmacytoid DC, B-cells</th>
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<tbody>
<tr>
<td>Natural Ligand</td>
<td>ssRNA</td>
<td>ssRNA</td>
<td>Bacterial DNA</td>
</tr>
<tr>
<td>Small Molecule Agonist</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>IL-12 induction</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>TNFα induction</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>IFNα induction</td>
<td>No</td>
<td>Yes</td>
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Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Can Augment Tumor Cell Killing
Phase 1b Head & Neck Trial: VTX-2337 + cetuximab

Representative Flow Cytometry Data
First-line therapy in locally advanced and metastatic head and neck cancer

- SOC: platinum, 5-FU, cetuximab
- 477 patients cisplatin (approx 60%) or carboplatin
- Primary Tumor sites:
  - oropharynx
  - hypopharynx
  - larynx

**Kaplan-Meier Estimates of Overall Survival and Progression-free Survival According to the Treatment Group**

<table>
<thead>
<tr>
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<th>PF + cetux</th>
<th>PF</th>
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<tbody>
<tr>
<td>OS</td>
<td>10.1 m</td>
<td>7.4 m</td>
</tr>
<tr>
<td>PFS</td>
<td>5.6 m</td>
<td>3.3 m</td>
</tr>
<tr>
<td>ORR</td>
<td>36%</td>
<td>20%</td>
</tr>
<tr>
<td>Disease Control</td>
<td>81%</td>
<td>60%</td>
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Hazard ratio (95% CI): 0.80 (0.64–0.99)  
P=0.04

Hazard ratio (95% CI): 0.54 (0.43–0.67)  
P<0.001

NEJM 2008:359:1116
VTX-2337 enhanced cetuximab-dependent degranulation of NK cells

%CD107a+ NK cells

**

Not Antibody

Cetuximab

Untreated

VTX 250nM

VTX 500nM

IL-2
TLR8 stimulation (VTX-2337) May Enhance T cell priming Effects of mAb Response

- **ADCC**
  - Effector cell
  - Cetuximab coated EGFR
  - Cytolysis

- **Cross-presentation of tumor antigens by DC**
  - TLR8+ myeloid Dendritic Cell
  - Internalization of Ab-coated TA and cross presentation to T cells
  - T cell stimulation and expansion

- **Cetuximab**
  - Fc
  - (ab)

- **EGFR+ tumor cell**
In vitro stimulation of EGFR-specific T cells is enhanced by VTX-2337 stimulated, cetuximab-treated NK:DC

![Graph showing the enhancement of EGFR-specific CD8 T cells/10,000 over time.](image-url)
Active-8 trial: TLR8 adjuvant to enhance Cetuximab-based immunotherapy

Screening ≤ 14 days

Randomization

Initial Treatment 6 Cycles (18 Weeks)
- Cisplatin/Carboplatin, 5-FU, and Cetuximab + VTX-2337
- Cisplatin/Carboplatin, 5-FU, and Cetuximab + Placebo

Subsequent Treatment 7+ Cycles
- Cetuximab + VTX-2337
- Cetuximab + Placebo

Follow Up
- Disease Progression
- Survival Follow Up

Standard of care consists of platinum chemotherapy (cisplatin or carboplatin), 5-FU, and cetuximab

PI's- Cohen and Ferris
Targeting model to restore type 1 microenvironment - reversal of tumor immune escape and CTL recognition of HNC

RANTES & IP10 chemokine secretion

Chemoattraction into TME

Reprogramming of anti-tumor CTL to reverse inhibitory signals

Anti-CTLA4
Anti-PD1

Tumor cell death

TA processing
HLA class I antigen presentation
Lab members

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Andrés López-Albaiteror, MD

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