Gene Therapy of liver cancer: Experience from clinical trials

Falk Symposium
Freiburg
October 2006

Division of Hepatology and Gene Therapy.
Center for Applied Medical Research (CIMA)
University Clinic. University of Navarra
Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 67% (n=762)
- Monogeneic diseases 8.7% (n=100)
- Vascular diseases 8.7% (n=100)
- Infectious diseases 6.6% (n=75)
- Other diseases 3.2% (n=37)
- Gene marking 4.5% (n=52)
- Healthy volunteers 1.7% (n=19)
Phases of Gene Therapy Clinical Trials

- Phase I: 62% (n=714)
- Phase I/II: 20% (n=234)
- Phase II: 14% (n=161)
- Phase II/III: 1% (n=12)
- Phase III: 2.1% (n=24)
Viral Vectors for Gene Transfer

Short-term expression vectors
- First generation adenovirus

Replicative cytopathic vectors
- Conditioned replicating adenoviruses

Long-term expression vectors
- Gutless or helper-dependent adenoviruses
- Retrovirus (lentivirus)
- Adeno-associated viruses (AAV)
Gene Therapy of Cancer

- Reversing the malignant phenotype
- Interfering with tumor biology:
  - antiangiogenic intervention
  - blocking survival signals
- Molecular chemotherapy
- Genetic immunotherapy
Treatment of established HCC with Ad.II-12

Before treatment

Ad.II-12

Ad.lacZ

Barajas et al HEPATOLOGY 2000
University of Navarra Clinical Trial of Ad.IL12 in GI Tumors

- Phase I, non-controlled design
- 21 patients with advanced GI tumors
  - Hepatocellular Carcinoma, Liver Metastases of Colorectal Cancer or Pancreatic Cancer
- Intratumor injection of Ad.IL12 (1st-generation, non-replicative adenovirus)
- 3 monthly injections of the same dose
- Dose escalation from $2.5 \cdot 10^{10}$ vp to $3 \cdot 10^{12}$ vp
- Main goals: feasibility and safety

Sangro et al. Journal Clinical Oncology, 2004
Echo-guided gene therapy of HCC with Ad.IL-12: Needle at the edge of the lesion
Changes after therapy (pg/ml)

* p < 0.05 vs. day 0

IL12/00/001
IFN-γ Induction

Journal Clinical Oncology, 2004
IL12/00/001
Tumor Infiltration by CD8+ T Cells

Patient 09/ Tumor: HCC/ Vector dose: $2.5 \cdot 10^{11}$ pv

Before Therapy

After Therapy

Journal Clinical Oncology, 2004
### IL12/00/001: antitumor response

<table>
<thead>
<tr>
<th>Dose step</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</table>

**Tumor** - **P**: pancreas; **H**: hepatocellular carcinoma; **C**: colorectal; **Cc**: cholangiocarc.

**Respuesta** - **Pr**: progression; **S**: stabilization; **P**: partial response

**Status** - **D**: death; **A**: alive

*Journal Clinical Oncology, 2004*
IL12/00/001: antitumor response
(patient 09 with HCC; retroperitoneal metastatic lymph nodes)

Before therapy

After 3 doses of Ad-IL12

Journal Clinical Oncology, 2004
Gene therapy of HCC with Adenovirus encoding thymidin kinase (HSV-tk)

- Phase I/II
- Dose escalation ($10^{11}$- $3 \times 10^{12}$ vp)
- 3 monthly intratumoral injections
GENE THERAPY WITH ADENOVIRAL VECTORS CONTAINING THYMIDIN-KINASE

Ad-tk

2 days

GCV x 14 days

Antitumor effect

Necrosis, apoptosis, inflammation
PET monitoring of tk activity (using $^{18}$FHBG as substrate for tk) in rats injected with Ad.tk or Ad.LacZ
DAY 0

AdCMVtk

DAY 2

PET

Penuelas et al Gastroenterology, 2005
Ad.tk, $10^{12}$ vp

Gastroenterology, 2005
First generation adenoviral vectors:

- given by i.t. injection are well tolerated and infect very efficiently HCC tumor nodules
- however, duration of the expression is very short, repeated tumor transduction is not possible and antitumor efficacy is very limited.
TUMOR MALIGNANT CELLS

DIRECT ULTRASOUND-GUIDED INJECTION

TH CTL PRECURSOR

NKs

DIFFERENTIATION

IN VITRO

PERIPHERAL BLOOD OR LEUKOAPHERESIS PRODUCT

VIRAL VECTOR ENCODING IMMUNOSTIMULATING FACTOR

LYMPH NODE

MALIGNANT CELL AG TRANSFER MIGRATION

EFFECTOR CTLs

CTL PRECURSOR

NKs

TRANSECTED CYTOKINE
SYSTEMIC THERAPEUTIC IMMUNITY

Non-treated side

Mean tumor diameter (mm)

DC AdCMVIL-12

Treated side

DC AdCMVLacZ

Week after tumor injection.
Leukapheresis

Tumor biopsy

PBMCs

DTH

1st dose

AFIL-12

DC culture

0

-1w

Leukapheresis

Tumor biopsy

PBMCs

CD14+ cells

DTH

Immune monitoring

Clinical response

2nd dose

AFIL-12

DC culture

3w

Thaw

DTH

Tumor biopsy

Immune monitoring

3rd dose

AFIL-12

DC culture

6w

Thaw

CD14+ cells

DTH

Immune monitoring

Follow-up

Safety evaluation

8w

Immunne monitoring

1 year

Journal Clinical Oncology 2005
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<th>Patient Num.</th>
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*: Concurrent chronic hepatitis C viral infection

Journal Clinical Oncology 2005
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<td>US</td>
<td>NE</td>
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</table>

PR: partial response
PD: progressive disease
SD: stable disease
NE: not evaluable
Variation in the number of tumor infiltrating lymphocytes following therapy

CD8+ lymphocytes

Journal Clinical Oncology 2005
A case of partial response to therapy

A) Before treatment

B) After treatment

Journal Clinical Oncology 2005
Sequestration of DCs inside the tumor
IL-8 serum concentration (pg/ml)

Patients Controls

\[ P < 0.001 \]

Tumor cell lines

ASPC-1 HEPG2 CaCo2 HT29 SW48

IL-8 (pg/ml)

~ 39 kDa

Neg NP iDC mDC

~ 40 kDa

Neg NP iDC mDC

CXCR1

CXCR2

International Journal of Cancer, 2006
Liver
Intrahepatic levels
Blood concentration

Long-term expression vector with therapeutic gene

Multifocal HCC
HCC in *PK/c-myc* transgenic mice

**Colony of *PK/c-myc* transgenics**

- *C-myc* heterozygous
- 1 month of age → carbohydrate-rich diet
- 8 months of age → 70% develop HCC

**normal mice**

**HCC in TG mice**

1 month of age → carbohydrate-rich diet
8 months of age → 70% develop HCC
Modified Tet-on regulatory system

Liver-specific constitutive promoter

EalbPa1AT → rtTA2s-M2

rtTA2s-M2 → Palb IL-12

+ Dox

P. Inducible

tetO7

Zabala y cols., Cancer Res, 2004
Repeated induction of IL-12 expression in the liver of PK/c-myc transgenic mice with multifocal HCC

Four treatment rounds and 3 cycles of induction each round

Laparotomy
Hydrodynamic injection of the plasmid

1ª Induction 2ª 3ª 3-5 weeks 4th ROUND

Day 0 Day 1 Day 51 y 112 Day 168
Laparotomy Laparotomy Sacrifice
Repeated induction of IL-12 expression in the liver of PK/c-myc transgenic mice with multifocal HCC: antitumor efficacy

**Tumor size and survival**

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<thead>
<tr>
<th></th>
<th>Saline</th>
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<td><strong>Tumor regression</strong></td>
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<td><strong>No response</strong></td>
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<td>6/10</td>
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<tr>
<td><strong>% Survival</strong></td>
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<td>70</td>
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*P<0.05*
Repeated induction of IL-12 expression in the liver of PK/c-myc transgenic mice with multifocal HCC: antitumor efficacy
Lymphocyte infiltration in c-myc transgenic mice subjected to repeated cycles of IL-12 induction in the liver
T cell-mediated immunosuppression

- The main obstacle to cancer immunotherapy

- T cell-mediated immunosuppression is executed by
  - Treg: CD4+ CD25+ Foxp3+ T cells
  - Tr1: CD4+ IL-10+ Foxp3- T cells
  - CD8+ IL-10+ regulatory T cells

- Treg in tumor microenvironment
  - Tumors induce Treg trafficking, differentiation and expansion
  - Treg block immune response against tumor-associated antigens
Regulatory T cells (Treg) and the suppression of antitumor immunity

Tumor infiltrating lymphocytes

Treg
CD4⁺CD25⁺
Induction of Foxp3 and IL-10 in IL-12-treated c-myc transgenic mice

**Foxp3**

- IL12 (R)
- IL12 (NR)
- Saline

**IL10**

- IL12 (R)
- IL12 (NR)
- Saline
TGFβ and T cell-mediated immunosuppression

- TGFβ is present at high levels in tumor microenvironment
- TGFβ plays a key role in the induction of Foxp3+ T reg
- TGFβ mediates the suppressive effect of regulatory T cells on adaptive immunity and NK cell function
Tumor volume after 10 days induction of IL-12 expression in the liver of rats with metastatic colon cancer: The effect of TGFβ blocking peptide P17
Animal Survival

% survival

Days

- Control
- GL-IL12
- GL-IL12+P17
Double inducible system in a single vector
Aims in cancer gene therapy

1. To increase the duration of transgene expression

2. To control transgene expression using inducible promoters

3. To identify the effective transgene or combination of transgenes to achieve curative antitumor effects

4. To monitor transgene expression by in vivo imaging techniques
NIL. DIFFICILE VOLENTI
# Collaborators

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