Translating Research into Clinical Practice: Strategies Against Hepatocellular Cancer

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The 20 Most Commonly Diagnosed Cancers Worldwide, 2008 Estimates

- Lung (13%) - 1,608,055
- Female Breast (11%) - 1,384,155
- Colorectum (10%) - 1,235,108
- Stomach (8%) - 988,602
- Prostate (7%) - 899,102
- Liver (6%) - 749,744
- Cervix (4%) - 530,232
- Oesophagus (4%) - 481,645
- Bladder (3%) - 382,660
- Non-Hodgkin Lymphoma (3%) - 356,431
- Leukaemia (3%) - 350,434
- Uterus (2%) - 288,387
- Pancreas (2%) - 278,684
- Kidney (2%) - 273,518
- Lip and Oral Cavity (2%) - 263,020
- Brain and CNS (2%) - 237,913
- Ovary (2%) - 224,747
- Thyroid (2%) - 213,179
- Malignant Melanoma (2%) - 199,627
- Larynx (1%) - 150,677
- Other Sites (12%) - 1,566,634

Cancer Research UK 2011
The 20 Most Common Causes of Death from Cancer Worldwide, 2008 Estimates

- Lung (18%) - 1,376,579
- Stomach (10%) - 737,419
- Liver (9%) - 695,726
- Colorectum (8%) - 609,051
- Female Breast (6%) - 458,503
- Oesophagus (5%) - 406,533
- Cervix (4%) - 275,008
- Pancreas (4%) - 266,669
- Prostate (3%) - 258,133
- Leukaemia (3%) - 257,161
- Non-Hodgkin Lymphoma (3%) - 191,599
- Brain and CNS (2%) - 174,880
- Bladder (2%) - 150,282
- Ovary (2%) - 140,163
- Lip and Oral Cavity (2%) - 127,654
- Kidney (2%) - 116,368
- Gallbladder (1%) - 109,587
- Other Pharynx (1%) - 95,550
- Larynx (1%) - 81,892
- Uterus (1%) - 73,854
- Other Sites (13%) - 962,191
Currently-Available Treatment

1. Surgical Resection
2. Liver Transplantation
3. Radiofrequency Ablation (RFA)
4. Transarterial Chemoembolization and Radioembolization (TACE)
5. Chemotherapy - sorafenib, FDA approved 2006
High mortality rate

High rate of recurrence

Limited benefit of current therapies

Urgent need for new therapeutic approaches
Immunotherapy for HCC

A Promising Strategy

Emerging Hallmarks and Enabling Characteristics

Emerging Hallmarks

Deregulating cellular energetics

Avoiding immune destruction

Genome instability and mutation

Tumor-promoting Inflammation

Immunosuppressive mechanisms

- Kupffer cell
  - PD-L1
  - PD-1

- MDSC
  - IL-10

- Treg
  - IL-10

- DC

- Effector CD8+ T
  - PD-L1
  - PD-1
  - IL-10

- Tumor cell
  - TAA

- Helper CD4+

Failure to kill
HCC-Specific Antigens Avoid Immune Destruction

- Hepatocellular cancer (HCC) antigens:
  - AFP (Alpha Fetoprotein), Glypican 3 (GPC3), etc.

- Liver is a tolerogenic organ:
  - maintains immune tolerance to digested antigens
  - induces tolerance in liver transplant recipients
Clinically Relevant Murine model of HCC
Spontaneous Hepatocellular Cancer Model-MTD2

Strength

- C57BL/6 mice which are transgenic for the SV40 T-antigen
  -- Binds and inactivates tumor suppressor proteins p53 and RB
  -- Induces spontaneously arising tumors when expressed as a transgene
- Highly immunogenic with well characterized CD8+ T-cell response

Weakness

- Hepatocytes are all transgenic and potentially tumorigenic
- Rapid tumor progression, early death
- Difficulty monitoring response to therapy
- Central Tolerance
Combinational Strategy to Make Clinically Relevant Murine Model:

CCl$_4$ injection and Intrasplenic Inoculation of Oncogenic Hepatocytes
Liver Fibrosis
Tumor Initiation and Progression

Normal

Tumor-bearing mice

Early (small tumors) (61 mm³)

Advanced (large tumors) (410 mm³)

Control

CCL₄ (3 wks)

CCL₄ (6 wks)
MRI Monitors Tumor Initiation and Progression

-/- CCl₄

CCl₄ (3 wks)

CCl₄ (6 wks)
Tumor antigens

Expression of Tumor-Specific-Antigen (TSA)

Expression of Tumor-Associated-Antigen (TAA)
Immune Response in Tumor-bearing Mice

Tumor-specific T cells are turned off as the tumor grows.
Tumor growth induces the increase of Tregs

Regulatory T cell (Treg)

% of CD4^+CD25^+FoxP3^+

Normal mice  Tumor mice
Tumor growth induces the increase of MDSCs

Myeloid-derived suppressor cells (MDSCs)

% of CD4^+CD25^+FoxP3^+
Summary

- A high-fidelity model of HCC
- Mimics human HCC initiation and progression as tumor grows in the setting of liver fibrosis
- Reflects typical features of this disease process
- Tumors express clinically relevant tumor antigens
- Immune response to tumor antigen can be tracked as tumor progresses
A Synergistic Chemoimmunotherapeutic Approach for HCC

HEPATOLOGY 2012;55:141-152
Sunitinib

- A small molecule inhibitor of RTK, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, FLT3, KIT, RET.

- The drug for treatment of ccRCC and GIST granted by FDA in 2006

- Being investigated for treatment of breast cancer, colorectal cancer, non–small cell lung cancer, and hepatocellular cancer
The combination of sunitinib treatment with adoptive T cell transfer synergizes to promote HCC regression in tumor-bearing mice.
Sunitinib treatment blocks CD8$^+$ T-cell tolerance in tumor-bearing mice.
Sunitinib treatment reduces the magnitude of Tregs and MDSCs in tumor-bearing mice.
Sunitinib specifically inhibits the activation of STAT3 in HCC cells

Sk Hep1 (A) or HepG2 (B) cells were treated with sunitinib at indicated concentration for 24 h. Western blotting analyses indicated sunitinib dramatically inhibited the activation of STAT3.
STAT3 activation leads to immune tolerance

Hua Yu, Marcin Kortylewski and Drew Pardoll: NATURE REVIEWS
Summary:

Sunitinib inhibits HCC tumor growth directly through the STAT3 pathway and prevents tumor antigen-specific CD8 T-cell tolerance, thus defining a synergistic chemoimmunotherapeutic approach for HCC.
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RFA in Combination with Immunotherapy in the Treatment of HCC

A tissue-mimicking media for optimizing RFA conditions
Liver ablation of normal mice with RFA
H & E staining of liver tissue and the levels of ALT and AST in the blood of RFA-treated mice

A

W/O  
Sham  
RFA

Day 1

Day 7

B

1400
1200
1000
800
600
400
200
0

ALT (u/L)

Day 0  Day 1  Day 7  Day 14

RFA  Sham

C

1800
1600
1400
1200
1000
800
600
400
200
0

AST (u/L)

Day 0  Day 1  Day 7  Day 14

RFA  Sham
RFA Destroys Small and Large Tumor

A

Small tumor

Day 0

Day 14

Large tumor

B

Damaged area (mm²)

Day 0

Day 7

Day 14

C

Pre-RFA

Day 14

RFA

tumor

RFA

tumor
Characteristics of RFA-induced Tumor Damage

A

- RFA  7 Days Post RFA  14 Days Post RFA

Small Tumor

Liver

RFA

RFA

Tumor

Tumor

Large Tumor

Tumor

RFA

RFA

B

ALT (u/L)

AST (u/L)

Day 0  Day 1  Day 7  Day 14

RFA  Sham

RFA  Sham

C

ALT (u/L)

AST (u/L)

Day 0  Day 1  Day 7  Day 14

RFA  Sham

RFA  Sham
Monotherapeutic efficacy of RFA on small and large tumor-bearing mice

Impact of RFA on Immune Response in Large Tumor-bearing Mice
RFA does not significantly increase the frequency of TSA-CD8+ T cells
RFA does not increase the frequency of CD8 T cells secreting IFN-γ in response to stimulation with for Tag-epitope-I in tumor-bearing mice.
RFA impacts the frequency of CD8 T cells secreting TNF-α in response to stimulation with for Tag-epitope-I in tumor-bearing mice.
RFA impacts PD-1 expression in CD8 T cells from tumor-bearing mice
Ongoing RFA-based Immunotherapeutic Strategies

1. RFA in combination with Sunitinib for the treatment of HCC

2. RFA in combination with anti-PD-1 antibodies for the treatment of HCC

3. RFA in combination of sunitinib and anti-PD-1 antibodies for the treatment of HCC
Future Direction: Laser Ablation in Combination with Immunotherapies in the Treatment of HCC
Development of Laser Ablation for the Treatment of HCC

a) ISPL inoculation of hepatocytes from MTD2 mice → MRI to monitor tumor

b) Laser therapy → GC injection → Euthanization 30 days after LIT

Mouse 1  Mouse 2

c) HCC Mouse 1

44.1°C

HCC Mouse 2

45.3°C

d) Temperature (°C) vs Time (minute)

M1, M2, M3, M4, M5, M6, M7, M8, M9, M10
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