Emerging immunotargets and primary tumor-infiltrating lymphocytes as biomarker in metastatic renal cell carcinoma

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University Hospital Zurich

Innovation in Pathology

High Throughput Scanning

Fusion of genomics and pathology data

New "Imaging" Technologies

New Drugs

Machine Learning

Big Data Science

Expert/Crowd Sourcing

Giesen et al. Nat Methods 2014
Immunotargets in RCC

• VHL alteration, renal cancer progression and therapy

• VHL protein, microvessels, TAM and TIL

• Quantification of microvessels and TIL
Clinical facts on Renal Cell Carcinoma (RCC)

**Incidence**
2% of all cancers, 20-30% of patients are diagnosed with metastasis

**Mortality**
30-40% die due to the disease

**Treatment**

**Localized disease**
Surgery

**Advanced disease**
VEGF-targeted therapies or immunotherapy
Renal Cell Carcinoma (RCC) subtypes

- clear cell: 75%
- papillary type 1: 15%
- papillary type 2: 5%
- chromophobe: 5%

Genes for familial types:
- VHL
- MET
- FH
- BHD (FLCN)
The VHL-Proteine

- pVHL
- HIFα
- HIFα
- HIFα
- Rbx1
- Cul 2
- Elongin B
- Elongin C

O2 → VEGF ↑

O2
Sunitinib

Sunitinib versus Interferon Alfa in Metastatic Renal-Cell Carcinoma

Robert J. Motzer, M.D., Thomas E. Hutson, D.O., Pharm.D., Piotr Tomczak, M.D., M. Dror Michaelson, M.D., Ph.D., Ronald M. Bukowski, M.D., Olivier Rixe, M.D., Ph.D., Stéphane Oudard, M.D., Ph.D., Sylvie Negrier, M.D., Ph.D., Cezary Szczylik, M.D., Ph.D., Sindy T. Kim, B.S., Isan Chen, M.D., Paul W. Bycott, Dr.P.H., Charles M. Baum, M.D., Ph.D., and Robert A. Figlin, M.D.*

Motzer R 2007, NEJM
Sunitinib

Hazard ratio, 0.42; 95% CI, 0.32–0.54; P<0.001

No. at Risk

<table>
<thead>
<tr>
<th></th>
<th>375</th>
<th>235</th>
<th>90</th>
<th>32</th>
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<td>Sunitinib</td>
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<td>Interferon alfa</td>
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Motzer R 2007, NEJM
Emerging Immunotargets in Metastatic Renal Cell Carcinoma

John Kucharczyk\textsuperscript{a}, Marc R. Matrana\textsuperscript{b}, Matteo Santoni\textsuperscript{c}, Francesco Massari\textsuperscript{d}, Marina Scarpelli\textsuperscript{c}, Li-ang Cheng\textsuperscript{f}, Antonio Lopez-Beltran\textsuperscript{g}, Stefano Cascinu\textsuperscript{c}, Rodolfo Montironi\textsuperscript{c} and Moch Holger\textsuperscript{h}

Table 2. Ongoing clinical trials on PD-1/PD-L1 blocking agents (from clinicaltrials.gov).

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Target</th>
<th>Phase</th>
<th>Description</th>
<th>ClinicalTrials.gov identifier</th>
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<tr>
<td>Nivolumab</td>
<td>PD-1</td>
<td>III</td>
<td>Compare OS vs everolimus in advanced mRCC pretreated with anti-angiogenic therapy.</td>
<td>NCT01688784</td>
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<td>II</td>
<td>Alone vs combination with bevacizumab or ipilimumab on mRCC</td>
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<td>I</td>
<td>Combination with sunitinib, pazopanib or ipilimumab</td>
<td>NCT01472081</td>
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<td>III</td>
<td>PFS and OS in patients with untreated RCC with nivolumab/ipilimumab combination therapy vs sunitinib monotherapy</td>
<td>NCT02231749</td>
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<td>Dose-ranging study on RCC pretreated with anti-angiogenic therapy</td>
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<td>Biomarker analysis of mRCC patients receiving nivolumab therapy</td>
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<td>Pembrolizumab</td>
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<td>Neoadjuvant therapy in patients receiving RCC tumor resections</td>
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<td>Pembrolizumab +/- pazopanib in naïve advanced RCC patients</td>
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<td>Pembrolizumab + PegIFN-2b vs pembrolizumab + ipilimumab advanced melanoma and RCC</td>
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<td>I/II</td>
<td>Pembrolizumab + bevacizumab in mRCC</td>
<td>NCT02348008</td>
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<td>I</td>
<td>Pembrolizumab + ziv-alibibercept in advanced solid tumors including RCC</td>
<td>NCT02298959</td>
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<td>Pidilizumab</td>
<td>PD-1</td>
<td>II</td>
<td>Alone or with DC/RCC fusion cell vaccine</td>
<td>NCT01441765</td>
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<td>MPDL3280A</td>
<td>PD-L1</td>
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<td>As monotherapy vs combination therapy with bevacizumab or sunitinib in untreated advanced RCC</td>
<td>NCT01984242</td>
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<td>I</td>
<td>First in human study in advanced and metastatic RCC</td>
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<td>Combination with ipilimumab or IFN α-2b in advanced/metastatic RCC</td>
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<td>BMS-936559</td>
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<td>I</td>
<td>Multidose study in advanced solid tumors including RCC</td>
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</table>
Renal Tumors

Diagnostic and Prognostic Biomarkers

Puay Hoon Tan, MD, FRCPA,* Liang Cheng, MD,† Nathalie Rioux-Leclercq, MD,‡ Maria J. Merino, MD,§ George Netto, MD,¶ Victor E. Reuter, MD,¶¶ Steven S. Shen, MD,##
David J. Grignon, MD,† Rodolfo Montironi, MD, FRCPH,*** Lars Egevad, MD,††
John R. Srigley, MD, FRCPC,‡‡ Brett Delahunt, MD, FRCPA,§§ Holger Moch, MD,|||
and The ISUP Renal Tumor Panel

Am J Surg Pathol 37, 2013

No Predictive Biomarker for Therapy Response
Immunotargets in RCC

• VHL alteration, renal cancer progression and therapy

• VHL protein, microvessels, TAM and TIL

• Quantification of microvessels and TIL
Molecular Pathways and Targeted Therapies in Renal-Cell Carcinoma

PD-L1 expression is regulated by HIF in clear cell RCC

Ruf et al.: Int J Cancer March 2016
Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma

Stefanie Regine Dannenmann,1 Julia Thilicke,1 Martina Stöckli,1 Claudia Matter,1 Lotta von Boehmer,1 Virginia Cecconi,1 Thomas Hermanns,2 Lukas Hefermehl,2 Peter Schraml,3 Holger Moch,3 Alexander Knuth1 and Maries van den Broek1,*

- RCC attract TAM
- enhanced expression of PD1
- association with brain mets

Brain metastasis in renal cancer patients: metastatic pattern, tumour-associated macrophages and chemokine/chemoreceptor expression

L Wyler3, C U Napoli2, B Ingold1, T Sulser2, M Heikenwälder4, P Schraml1 and H Moch1,*

1Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland; 2Institute of Pathology, University Hospital Basel, Basel, Switzerland; 3Clinic for Urology, University Hospital Zurich, Zurich, Switzerland and 4Institute of Virology, Technische Universität München (TUM), Helmholtz Zentrum München (HMGU), München, Germany
Immunotargets and TIL in RCC

• VHL alteration, renal cancer progression and therapy

• VHL protein, microvessels, TAM and TIL

• Quantification of microvessels and TIL
Relevance of Periostin Splice Variants in Renal Cell Carcinoma
Evaluation of Microvessel Area by AQUA-System

Automated Quantitative Analysis; McCabe et al.: JNCI 97, 2005:

Mertz et al.: Epithelial/Stromal-Mask (CD10; EMA; Vimentin)
Evaluation of Microvessel Area by AQUA-System

MVA is associated with Survival

Survival Functions

\[ P < 0.0001 \]

Mertz et al.: Hum Pathol 2007
Vessel Segmentation, Classification and Quantification by Tissue Studio and Developer XD

- Tissue Studio: Vessel Extraction and Classification
- Developer XD: Tissue Classification in Tumor and Non Tumor Regions
TissueStudio
Blood vessel density (CD34)

Overall survival (%, x100)

Time

N=27

N=23

p=0.0368
Peritumoral lymph vessels

D2-40
D2-40
Intra- and peritumoral lymph vessels!
Intratumoral lymphatic vessel density (LYVE1)

- Mean
  - N=35
  - N=12

- Median
  - N=25
  - N=22

Overall survival (% x100)

Hemato-Lymphangiogenic Switch
Results

• high MVD of blood vessels associated with better prognosis
• Peritumoral lymph vessels (PTL) not associated with prognosis
• Intratumoral lymph vessels (ITL) associated with poor outcome
• ITL associated with high TIL count
• ITL or PTL associated with tertiary lymphatic structures?
Future Projects

• Quantification of TIL
• Quantification of TAM
• Quantification of peritumoral TLS
  – Lymphatic aggregates w/wo germinal centers
  – Tertiary lymphatic structures
• Correlation of TIL, TAM and TLS with prognosis and therapy response
• Co-registration of different biomarkers in RCC (CD70-positive RCC and CD27-positive TIL)
Quantitative pathology approach for quantification of TILs

1. Multi-colour IF staining (Opal TSA kit)
   Ventana Discovery

2. Multispectral imaging of slides
   Vectra 3.0

3. Un-mixing of fluorescent signals
   InForm software

4. Training of cell phenotype algorithm
   InForm software

5. Quantification of different cell phenotypes
   InForm software

6. Statistical analysis
   MS Exel, SPSS
Patient ID: 06-24784
Image ID: TLS_[42861,9265]
Tumour IF: CD3 CD20 Lyve1
Patient ID: 06-24784
Image ID: TLS_[42861,9265]
Cell phenotyping by Inform software: CD3 CD20
Lyve1 Other
Patient ID: 06-24784
Image ID: TLS_[45214,11661]
TLS in tumour periphery IF: **CD3** **CD20** **Lyve1**
Tertiary Lymphatic Structures TLS (mm$^2$)

Overall survival (% x 100)

N=11
N=38

p=ns
CD70 is frequently expressed in primary ccRCC and metastases

**Clinical Trials:**

anti-CD70 agents (antibody-drug conjugates):

- MDX-1203 (NCT00944905)
- MDX-1411 (NCT00656734)
- SGN-75 (NCT01015911)
Co-Registration: CD27+ TIL preferentially infiltrate CD70-expressing RCC
PBMCs trigger the release of sCD27 in a CD70-dependent manner

- CD70 expression is regulated by HIF
- Increased serum levels of CD27 suggest existence of CD70-expressing ccRCC
- sCD27 as potential serum marker

Ruf et al.: Clin Cancer Res 2015
TIL: Lymphocyte Segmentation, Classification and Quantification by Developer XD

• First Step: applying Definiens Image Analysis solution for Nuclei Object Detection (developed by Dr. Nicolas Brieu).
  • Nuclei Objects are the different types of lymphocytes: CD3, CD8 and CD20

• Second Step: embedding lymphocytes into tumor and non-Tumor regions
Tissue Phenomics Applications

• Virtual multiplexing
  • Co-registration of images from different biomarkers
  • Analysis of relations between specific biomarkers and corresponding objects

• Statistics of mutual relations:
  • Tumor heterogeneity and distribution of lymphatic vessels (ITL, PTL) and bloodvessels (MVD)
  • Mutual relation of the local lymphatic vessel distribution to the local blood vessel distribution
  • Relative position of blood vessels and lymphatic vessels to TIL, TAM and peritumoral tertiary lymphatic structures (TLS)
CD8
Conclusion

- need of predictive biomarker for immunotherapy in RCC
- Potential vessel biomarker: MVD, ITL, PTL
- Potential lymphocyte biomarker: TIL, TAM and peritumoral TLS
- Existence of intratumoral lymphatics (ITL) is associated with poor prognosis
- Tissue Phenomics allows quantification of complex morphological structures
- Tissue phenomics as a tool to identify mutual relations between different biomarkers