Developing Magsifter & NIA to Measure Resistance to Targeted Therapy in CTCs

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Dilemma of measuring resistance to targeted therapy in renal cell carcinoma (RCC)

1. resistance mechanisms not well-understood
2. serial biopsies of tumor masses not feasible
3. Lack of sensitive, clinically validated protein signaling assays for clinical samples

Empiric selection

How do I know it’s working?

- 10 targeted therapies
- 7 agents against VEGF pathway
- 2 growth inhibition drugs
- 1 immune modulator

Tumor Initiation  Diagnosis  Treatment  3 months

CT scan

Response  Resistance
Dilemma of measuring resistance to targeted therapy in RCC

Tumor Initiation | Diagnosis | Treatment until progression | Clinical Outcome

Response

Resistance

measure resistance earlier
Improving patient outcomes to targeted therapy in RCC

- **Responders**
  - Reduce unnecessary toxicities
  - Potentially increase time to tumor resistance (or PFS)
  - Better tumor burden control
- **non-responders**
  - No benefit

**Benefits of “early failure” panel:**
- Reduce unnecessary toxicities
- Potentially increase time to tumor resistance (or PFS)
- Better tumor burden control
Project proposal

**Hypothesis**: Magsifting technology can be adapted to capture RCC circulating tumor cells (CTCs) and to measure resistance to targeted therapy in CTCs using nanoimmunoassay (NIA)

- **Aim 1**: Develop magnetic sifter technology to isolate CTCs from patients with RCC based on CAIX surface protein expression

- **Aim 2**: Profile RCC CTCs using nano immunoassay (NIA) to measure biologic resistance to drug therapy in VEGFR pathway, cell cycle and apoptosis proteins
CTCs are optimal cells to measure biologic resistance to targeted therapies

- shed from primary tumor & metastases
- circulates in blood stream —> ”liquid tumor”
- accessible for serial sampling —> ”liquid biopsy”
- circumvents inability of ctDNA to capture gene loss
- best candidate cell type to measure resistance to drug therapy

adapted from abnova.com
Project proposal

**Hypothesis**: We propose a novel approach to detect early therapeutic failure by assessing early signaling changes in circulating tumor cells (CTCs) before changes in tumor size are detectable by standard imaging criteria at 3 months.

- **Aim 1**: Develop magnetic sifter technology to isolate putative CTCs from patients with RCC based on CAIX surface protein expression

- **Aim 2**: Profile RCC CTCs using nano immunoassay (NIA) to measure biologic resistance to drug therapy in VEGFR pathway, cell cycle and apoptosis proteins
Patient blood can be magnetically “sifted” to isolate CTCs

CA9: Carbonic Anhydrase 9
(RCC cell surface marker)

Expansion from lung studies: Park, SM. PNAS. (2016)
Schematic diagram of magnetic “sifting” for CTC isolation

Earhart CM et al, Lab on a Chip (2014)
“Magsifter” processes multiple samples in parallel
“Magsifted” cells from mRCC patients are distinct from WBCs by immunofluorescence.

Capture yield: ~60%
Purity: ~30%

Work in progress: confirmatory immune staining of RCC-specific markers (PAX8)

Legend
Green: Pan-cytokeratin
Red: CD45
Blue: DNA
Pilot project to detect early biomarkers of resistance to targeted therapy

**Patients:**
N= 20

**Drug:**
pazopanib or sunitinib [TKI]

**Profile:**
magsifted cells before starting TKI, 2w, 6w & 3m
Hypothesis: We propose a novel approach to detect early therapeutic failure by assessing early signaling changes in circulating tumor cells (CTCs) before changes in tumor size are detectable by standard imaging criteria at 3 months.

• **Aim 1:** Develop magnetic sifter technology to isolate putative CTCs from patients with RCC based on CAIX surface protein expression

• **Aim 2:** Profile RCC CTCs using nano immunoassay (NIA) to measure biologic resistance to drug therapy in VEGFR pathway, cell cycle and apoptosis proteins
Nanoimmunoassay (NIA) is a novel proteomic platform for small clinical samples

Protein signatures can identify downstream molecular alterations in RCC

Candidate biomarkers of resistance

MAPK/ERK; PIK3CA/AKT

cleaved caspase 3

Ki-67, p15, p21
NIA can identify & quantify biologic response in “magsifted” cells vs PBMCs

**Work in progress:**
- optimizing magsifting pipeline to reliably measure ERK signaling
- in parallel, assay development for other markers in the panel
Improving patient outcomes to targeted therapy in RCC

% survivors

0 3 6 9 12 15 18

PFS (months)

% survivors

100 80 60 40 20 0

RESPONDERS

NON-RESPONDERS

initiate early, effective therapy

Standard CT scan
Summary

• “Liquid biopsies” are needed to track patients’ therapeutic status on TKIs

• ‘MagSifter’ is a viable strategy to isolate putative CTCs from blood draws

• NIA can profile biologic response of sifted patient cells before & during therapy

• Biomarker panel for early failure reduces unnecessary side effects and may even lead to increased PFS or OS
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Thanks for your attention!
The End
Plan to detect early clinical biomarkers

Patients: N= 20

Drug: pazopanib or sunitinib

phase I:
Profile magsifted cells 2 wks after starting TKI & at 3 months

phase II:
Profile at serial time points until median PFS reached for both TKIs to ensure a profile predictive of therapy failure
NIA signals in CDX mouse models