Overview of mTOR

- **Mammalian Target of Rapamycin**
- **Rapamycin:**
  - Product from *Streptomyces hygroscopicus*
  - Found in earth of Easter Island (“Rapa Nui”)
- Serine/threonine kinase
- Central role in PI3K/AKT/mTOR pathway
PI3K/AKT/mTOR pathway

- mTOR complex 1: mTOR + raptor:
  - Stimulated by insulin, growth factors, serum, amino acids (particularly leucine), and hypoxia
  - Target of rapamycin

- mTOR complex 2: mTOR + rictor:
  - Stimulated by insulin, growth factors, serum, and nutrient levels
  - Negative feedback loop via Akt

Courtesy of Konings I, Erasmus MC, Rotterdam, The Netherlands
mTOR activation in tumors

- Activated pathway results in:
  - Cell proliferation
  - Cell survival
  - Angiogenesis
  - Resistance to antitumor therapy

- Activation in tumors through:
  - Excess of ligand
  - Receptor overexpression
  - Gain of function mutations
  - PI3K or AKT amplification
  - Mutated PTEN
  - TSC1/TSC2 alterations
  - Nutrients / MAP4K3
  - p53 mutations

Courtesy of Konings I, Erasmus MC, Rotterdam, The Netherlands
Rapamycin and Rapalogs

- **Rapamycin:**
  - Initially developed as anti-fungal drug
  - Immunosuppressant organ transplantations:
    - Decrease in occurrence of malignancies:
      - Exploration as anti-tumor agents
      - Development of rapalogs:
        » More favorable pharmaceutical properties such as solubility and stability
mTOR inhibitors (aka rapalogs)

Rapamycin

Temsorilimus

Everolimus

Ridaforolimus

Sleijfer S, Erasmus MC, Rotterdam, The Netherlands
Preclinical data

Suppression of tumor growth by both daily and intermittent dosing schedules of RAD001

With permission from American Association for Cancer Research, Boulay A, et al., Cancer Res 2004;64:252-261
Establishing recommended dose for further studies

- Does dose and/or schedule matter for rapalogs?
  - MTD or lower “optimal biological dose” (equally effective but less toxic)

- Requires comparison between several doses in terms of:
  - Toxicity profile
  - Pharmacokinetics
  - Pharmacodynamics
  - Anti-tumor activity

- Of note:
  - Most relevant PK parameter not known; no clear dose-response relationship
  - Most relevant PD marker not known
Establishing recommended dose for further studies

- **Sirolimus:**
  - No traditional Phase I in cancer patients:
    - Doses: 1-8 mg daily adjusted on patient tolerance or trough levels (5-15 ng/ml)

- **Temsirolimus:**
  - Phase I with doses from 1.5 mg/m2 – 220 mg/m2 weekly iv:
    - No DLTs
    - Exposure active metabolites increased less than proportionally with increasing dose
    - no improved variability by dosing based on BSA over flat dosing
  - Randomized Phase II with temsirolimus in RCC:
    - 25, 75, 250 mg iv weekly:
      - No differences in RR, PFS, and OS
      - 25 mg further explored
      - However, not adequately powered to assess differences (i.e., 6 mths longer median OS at 250 mg not statistically significant)
Establishing recommended dose for further studies

- **Ridaferolimus (deforolimus):**
  - MTD identified
  - At RP2D:
    - Inhibition of phosphorylated 4eBP1 in platelets:
      - Adequate PD marker?

- **Everolimus:**
  - Phase I with weekly and daily doses:
    - MTD identified
  - More profound inhibition PD marker at RP2D than at lower doses:
    - Adequate PD marker?
  - Randomized Phase II breast cancer daily 10 mg vs. weekly 70 mg:
    - Daily 10mg: predefined RR for activity

With permission from American Society of Clinical Oncology, Tabernero J, et al., JCO, 2008; 26 (10):1603-1610
## mTOR inhibitors

<table>
<thead>
<tr>
<th>mTOR inhibitor</th>
<th>Dosing scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>rapamycin/Sirolimus</td>
<td>2-6 mg/day orally</td>
</tr>
<tr>
<td>temsirolimus/CCI-779</td>
<td>75 mg/day orally QDx5 every 2 weeks OR 25-250 mg/week IV</td>
</tr>
<tr>
<td>everolimus/RAD001</td>
<td>50-70 mg/week orally OR 10 mg/day orally</td>
</tr>
<tr>
<td>ridaforolimus/AP23573</td>
<td>12.5 mg QDx5 every 2 weeks IV OR 12.5 mg/week IV</td>
</tr>
</tbody>
</table>
Toxicity profiles

- No head to head comparison between different mTOR-inhibitors
- No apparent differences in toxicity profile

- Typical mTOR-inhibitor-associated toxicities:
  - Gastrointestinal
  - Dermatological
  - Mild myelosuppression
  - Elevated liver function tests
  - Dyslipidemia
  - Fatigue
  - Hyperglycemia
  - Hypophosphatemia
  - Asthenia/psychiatric
  - Allergic drug reaction
  - Pneumonitis

Toxicity profiles

- Mostly grade 1/2 toxicities:
  - Most common grade 3/4 toxicities: anemia, stomatitis, diarrhea, hyperglycemia

- Emerging toxicity with more common use: pneumonitis
  - Often not reported in early clinical trials
  - Incidence in placebo-controlled everolimus Phase III study in RCC:
    - Gr 1 (asymptomatic): 3.3%
    - Gr 2 (not interfering daily life): 6.6%
    - Gr 3 (interfering daily life/O2 dependent): 3.6%
    - Gr 4 (life-threatening): 0%

- Outcome (37 pts):
  - Completely recovered: 20/37
  - Unknown/died of PD: 15
  - Fatal: 2/37 pts with pneumonitis

Numerous Phase II trials in broad range of tumor types:
- Difficult to put into perspective: Non-randomized

Clinical Trials.gov:
- Currently no Phase III trials with monotherapy ongoing

Several Phase III trials published
Temsilirimus in poor-prognosis Renal Cell Carcinoma (RCC)

- Temsirolimus 25 mg iv. Weekly
- Comparison to IFN and IFN/tem
- Overall survival 10.7 vs. 7.3 months
- FDA-approval poor prognosis mRCC

Everolimus 2\textsuperscript{nd}/3\textsuperscript{rd} line after VEGFR-TKI in RCC

- PD under VEGFR-TKI
- Placebo-controlled Phase III, double blind; 2:1
- Only clear-cell RCC
- Everolimus 1dd 10 mg (oral)

\textit{Progression-free survival}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{progression_free_survival}
\end{figure}

With permission from Elsevier, Motzer RJ, \textit{et al.}, \textit{The Lancet} 2008; 372 (9637): 449-456
Everolimus 2\textsuperscript{nd}/3\textsuperscript{rd} line after VEGFR-TKI in RCC

Low RR:
- 1 vs. 0%

No OS benefit

Sleijfer S, Erasmus MC, Rotterdam, The Netherlands
Everolimus 2\textsuperscript{nd} /3\textsuperscript{rd} line after VEGFR-TKI in RCC

\textit{Time to 10\% KPS decline}

With permission from John Wiley & Sons, Inc, Motzer RJ, et al., Cancer, 2010;116(18):4256-4265
mTORC1 activation via IGF1
Pancreatic Neuroendocrine Tumors

- 410 patients, randomized to either Oral Everolimus 10 mg daily, or placebo

With permission from Massachusetts Medical Society, Yao JC, et al., NEJM 2011; 364: 514-523
Also presented by Yao JC, et al. at the 35th ESMO Congress 2010; Milan, Italy; Abstract LBA9
Ridaforolimus in soft tissue sarcomas

- Patients with metastatic disease
- CR, PR or SD after ≥ cycles of first line chemotherapy

- 711 patients randomized
- 552 PFS events
- 28% reduction in progression by ridaforolimus (hazard ratio=0.72) \( (P=0.0001) \)
- 21% improvement (3.1 week) in median PFS (ridaforolimus, 17.7 weeks vs. placebo, 14.6 weeks; statistically significant)

Chawla SP, et al, ASCO 2011; Abstract 10005
Combination regimens

- mTOR inhibitors
- Conventional cytotoxics
- Mabs
- Hormonal
- TKIs
- Radiotherapy
mTOR-containing regimens

- Activation of PI3K/AKT/mTOR pathway involved in resistance in multiple preclinical models

- Numerous Phase I trials:
  - Often difficult to combine:
    - Overlapping toxicity, particularly with TKIs
    - Drug-drug interaction?

- Randomized studies:
  - Phase II breast cancer: letrozole ± everolimus; tamoxifen ± everolimus
  - Phase III poor prognosis RCC: temsirolimus + IFN (discussed earlier)
  - Phase III advanced neuro-endocrine tumors: octreotide LAR ± everolimus
Breast cancer: hormonal treatment with mTOR-inhibitor

- HER1/2-induced PI3K/AKT/mTOR pathway activation yields resistance against hormonal agents
- Letrozole/placebo vs. letrozole/everolimus (Baselga et al., JCO 2009):
  - Neo-adjuvant breast cancer
  - Post-menopausal women
  - Primary endpoint: objective response:
    - Higher RR for combination by:
      - Clinical palpation (68 vs. 59%)
      - Ultrasound (58 vs. 47%)
      - Mammography (36 vs. 39% ; ns)
Breast cancer: hormonal treatment with mTOR-inhibitor

- Number of cases with <1% Ki-67+ cells in tumor:
  - Ki-67: proliferation marker
  - Suggested association of post-treatment values with relapse-free survival

*All patients, before and after Tx*
*More “successes” using combinations*

With permission from American Society of Clinical Oncology,
Breast cancer: hormonal treatment with mTOR-inhibitor

- Tamoxifen/placebo vs. tamoxifen/everolimus:
  - Metastatic breast cancer
  - PD under prior aromatase-inhibitor:
    - Enrichment for mTOR-activated patients?
  - Primary endpoint: Clinical benefit rate (RR + SD>6mnths):

With permission from Bachelot T; Bachelot T, et al., SABCS 2010; Abstract S1-6
Breast cancer: hormonal treatment with mTOR-inhibitor

Time to progression

- TAM: 4.5 mo.
- TAM + RAD: 8.6 mo.

Hazard Ratio (HR) = 0.53; 95% CI (0.35-0.81)
Exploratory log-rank: $P = 0.0026$

Overall survival

HR = 0.32; 95% CI (0.15-0.68)
Exploratory log-rank: $P = 0.0019$

With permission from Bachelot T, Bachelot T, et al., SABCS 2010; Abstract S1-6
Phase III: advanced neuro-endocrine tumors (NET)

- Advanced NET & carcinoid syndrome
- PD < 1 yr before study entry
- N=429

Sleijfer S, Erasmus MC, Rotterdam, The Netherlands
PFS Phase III: advanced neuro-endocrine tumors (NET)

**median PFS**
- Everolimus + Octreotide LAR: 16.4 months
- Placebo + Octreotide LAR: 11.3 months

HR = 0.77; 95% CI [0.59 - 1.00]

*P*-value = 0.026

Analysis based on Central Review

Pavel M, *et al.* 35th ESMO Congress 2010; AoO, ESMO LBA; 21 (8)

Courtesy of Novartis, Rare Tumors, Novartis Oncology-Region Europe
Biomarkers

- Biomarker for PI3K/AKT/mTOR pathway activation:
  - To determine optimal biologically active dose (OBD):
    - i.e. early clinical trials, to tailor drug doses in individual patient
  - Predictive marker

- Requirements for biomarkers:
  - Robust and validated assay
  - Associated with relevant endpoint:
    - i.e. early clinical trials: BM reflecting relevant anti-tumor effect
    - i.e. early clinical clinic: clinically relevant endpoint
  - Cost-effective (particularly for markers used in clinic)
Biomarkers

- Potential candidates to determine OBD:
  - Phosphorylation status downstream markers:
    - e.g. ↓ phosphorylation of pS6K, 4-EBP1, etc.
  - Anti-tumor effect in terms of:
    - Cellular level: apoptosis, Ki-67, etc.
    - Radiological means

- Potential candidates for predictive markers:
  - Causes of PI3K/AKT/mTOR pathway activation:
    - i.e. PTEN loss, PI3K mutation
  - Phosphorylation status downstream markers:
    - i.e. ↑ phosphorylation of pS6K, 4-EBP1

Courtesy of Konings I, Erasmus MC, Rotterdam, The Netherlands
Biomarkers to determine OBD

- i.e. phosphorylation status downstream markers frequently used:
  - Tumor, PBMCs, skin

- But: unknown whether:
  - Association with clinical relevant endpoint
  - Anti-tumor effects go via these factors
  - Optimal assay
  - Best site to do the test

- Questionable whether currently PD markers can be used for OBD

With permission from ASCO, Tabernero J, et al., JCO 2008; 26:1603-1610
Predictive factors

- Most promising: mutations yielding PI3K/AKT/mTOR pathway activation:
  - Preclinical models: high sensitivity to mTOR-inhibitors in tumor cells with PIK3 gain-of function mutations
  - In line with premature clinical data: letrozole ± everolimus in breast cancer

% cases with < 1% Ki-67+ cancer cells

Most profound effects for combination in mt PIK3CA?

With permission from ASCO, Baselga J, et al., JCO 2009; 27 (16): 2630-2637
Predictive factors

- TSC gene alterations as predictive factor?
  - TSC products inhibit mTOR-activity
  - Loss-of-function mutation in TSC yields mTOR activation

- Subependymal Giant-Cell Astrocytomas (SEGA) (NEJM 2010; 363: 1801-1811)
  - TSC mutations proven, mTORC1 activation hypothesized

- Perivascular epithelioid tumors (PEComas) (JCO 2010; 28: 835-830)
  - TSC changes in clinical samples with indirect evidence of mTORC1 activation
mTORC1 activation via TSC alterations
Subependymal Giant-Cell Astrocytomas (SEGA)

- 28 patients
- Oral Everolimus 3 mg/m² daily

Krueger DA, et al., NEJM 2010; 363:1801-1811
Graph by Sleijfer S, Erasmus MC, Rotterdam, The Netherlands
mTORC1 activation via TSC alterations
Perivascular epithelioid tumors (PEComas)

- Rare disease
- N= 3
  - Loss of TSC2 protein expression
  - Evidence of mTORC1 activation
- Sirolimus, 1 mg every other day – 8 mg daily, adjusted based on trough level and tolerance
- All patients showed lasting response

mTOR pathway involved in cellular survival, proliferation, angiogenesis, resistance to anticancer drugs

mTOR pathway frequently overactivated in tumors

mTOR inhibition promising in antitumor treatment

Four mTOR inhibitors currently available
  - Rapamycin, temsirolimus, everolimus, ridaforolimus

FDA-approval for:
  - Everolimus in mRCC, Subependymal Giant-Cell Astrocytomas (SEGA) associated with tuberous sclerosis, astrocytoma, Pancreatic NET
  - Temsirolimus: poor prognosis RCC
Many phase combination trials ongoing:
- Frequently difficult to combine

Need for valid biomarkers to identify optimal dose:
- Early clinical trials
- Dose tailoring

Need for valid predictive markers for response:
- Most promising: alterations in genes causing mTOR pathway activation (TSC, PIK3 mutations)
Thank you!