From IGF to mTOR Signaling in Pediatric Sarcomas—Opportunities for Novel Therapeutic Intervention
Role of IGF Signaling - Previous Studies

- **IGFII** is an autocrine growth and motility factor in rhabdomyosarcoma (El-Badry et al. Cell Growth and Diff 1990)

- Loss of Imprinting (LOI) of IGFII in Rhabdomyosarcomas (Zhan S, Shapiro DN, and Helman LJ JCI 1994)

- LOI of IGFII in Ewing’s Sarcoma (Zhan S, Shapiro DN, and Helman LJ Oncogene 1995)

- **IGFIR** is required for EWS-FLI-1 transformation of fibroblasts (Toretsky J. et al. JBC 1997)
In situ bright field IGF-II
In situ dark field IGF-II
• IGF-II overexpression in C2 myoblasts led to diminished G1 checkpoint (Zhang et al. JBC 1999)

• Resistance to apoptosis most directly correlated with phosphorylation of p70S6 kinase and 4E-BP-1. Resistance to apoptosis in IGF-II overexpressing cells was reversed by rapamycin (Wan and Helman Neoplasia 2002)
Cisplatin Decreases p70S6k Phosphorylation In Wild-type but not IGFII overexpressing C2 cells

Cells treated with 25μM CDDP for indicated times
Rapamycin Abolishes the Resistance of IGF-II Driven RMS Cells to CDDP Induced Apoptosis

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<th>Rh30-ARMS</th>
<th>CTR-ERMS</th>
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<td>Cisplatin</td>
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<td>Rapamycin + Cisplatin</td>
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Conclusions

• IGF signaling provides a survival signal that contributes to tumor cell resistance to DNA-damage induced cell death

• This resistance is associated with mTOR signaling, and can be reversed with agents that block mTOR
mTOR and Sarcomas


• Demonstrated that mTOR blockade with rapamycin or analogs inhibits RMS experimental pulmonary mets (Wan Cancer Res 2005)
Rapamycin and CCI 779 prolongs survival and inhibits pulmonary metastasis of K7M2 Osteosarcoma *in vivo*

Rapalogs and IGF Signaling

• Demonstrated that rapalog treatment of RMS leads to activation of Akt *in vivo*

• Demonstrated that this activation is IGF dependent and can be blocked with IGFIR blockade (*Wan et al. Oncogene* 2006)
Nutrients
GF (insulin/IGF)

RTK

IRS-1
PI3K

PI3P

PI3K

PKB/Akt

TSC1
TSC2
Rheb
mTOR
Raptor
mLST8
Rictor

AMPK

LKB1

Rapamycin
FKBP12

S6K1
4E-BP1

rpS6
eIF4B
eIF4E

Top-dependent translation (e.g. IGF-II)
Cap-dependent translation (e.g. Cyclin D1, c-MYC, HIF-1α, VEGF)

Actin cytoskeleton
In RMS, IGFIR levels directly correlate with sensitivity to IGFIR blockade \textit{in vitro}
IGFIR is responsible for the majority of Akt activation in RMS cell lines, and IGFIR Ab specifically downregulates IGFIR and pAkt in cell lines with high IGRIR levels.
Activated Akt reversed h7C10 effect on proliferation
In RMS, IGFIR levels directly correlate with sensitivity to IGFIR blockade *in vivo*
Uncoupling of IGFIR and Akt signaling after long-term Rx-RH30 cells. Combination IGFIRAb plus rapamycin is more potent in xenograft growth inhibition.
Nutrients
GF(insulin/IGF)

RTK

IRS-1
PI3K

PIP2
PIP3

PTEN

PDK1

PKB/Akt

TSC1
TSC2

Rheb

mTOR

Raptor

mLST8

Rictor

S6K1
4E-BP1

rpS6
elF4B

Top-dependent translation (e.g. IGF-II)

Cap-dependent translation (e.g. Cyclin D1, c-MYC, HIF-1α, VEGF)

Actin cytoskeleton

LKB1
AMPK

Rapamycin
FKBP12

Other RTK?
Conclusions

• Early evidence to suggest beneficial combination of mTOR inhibition combined with IGFIR inhibition
• Effect of IGFIR inhibition correlates with IGFIR levels
• Effect of IGFIR blockade on decrease in pAkt is lost in long-term xenografts, and this “tachphylaxis” is abrogated with mTOR inhibition
Clinical Studies
Refractory recurrent Ewing’s patient Rx with R1507
Refractory Ewing’s sarcoma patient Rx with R1507
44 year old US farmer with third systemic relapse of Ewing’s sarcoma
SARC Protocol #:  SARC 011
Hoffmann-La Roche Protocol #: N021157

TITLE: SARC Global Collaboration*: A Phase II Trial of R1507, a Recombinant Human Monoclonal Antibody to the Insulin-Like Growth Factor-1 Receptor for the treatment of patients with recurrent or refractory Ewing’s sarcoma, osteosarcoma, synovial sarcoma, rhabdomyosarcoma and other sarcomas

*SARC Global Collaboration represents collaboration among the Innovative Therapies for Children with Cancer (ITCC), Istituti Ortopedici Rizzoli, European Organization for Research (EORTC/STBG), Cooperative Osteosarcoma Study Group (COSS), EuroEwings, Euramos

Sponsor:                         F. Hoffmann-LaRoche

Coordinating Center:            SARC (Sarcoma Alliance for Research through Collaboration)
17 yo boy with multiply recurrent Ewing’s sarcoma

Baseline CT Chest  Week 6 CT Chest
Conclusions

• Humanized IGFIR moAb shows remarkable clinical activity in Ewing’s sarcoma patients in early Phase I studies
• Early evidence to suggest beneficial combination of mTOR inhibition combined with IGFIR inhibition
• Phase II study ongoing-responses ongoing
• Planned study using mTOR inhibitor plus IGFIR moAB
IGF-1R/InR → IRS-1 → PI3K → Akt → TSC1/2 → Rheb → mTORC1 ↔ mTORC2

IGF-1R Ab TK inhibitors

PI3K inhibitors

Akt inhibitors

Rapalogs

mTORC1 and mTORC2 inhibition with kinase inhibitors?
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<th>Lee Helman</th>
<th>Seth Cohen</th>
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<td>Chand Khanna</td>
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