A Phase 2 Study of Tarloxotinib Bromide (TRLX) in Patients with Recurrant or Metastatic Squamous Cell Carcinoma of the Head and Neck (SCCHN) or Skin (SCCS)

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Background

EGFR in SCCHN and SCCS

EGFR is a clinically-relevant molecular target for SCCHN and SCCS

- Expression of EGFR and its ligands is observed in >80% of SCCHN [1,2]
- High EGFR expression is associated with poor prognosis with SCCHN [3,4]
- EGFR overexpression is also reported with SCCS [5]

Single-agent EGFR-targeted therapies fail to substantially alter patient outcomes in recurrent SCCHN and SCCS

- Median progression-free survival (PFS) for relapsed/metastatic (R/M) SCCHN is generally less than six months and median overall survival (OS) is generally less than one year
- Mechanism-associated toxicities (diarrhea and skin rash) prevent administration of a dose sufficient to completely shut-down EGFR signaling in SCCHN tumors, leading to treatment failure [6]
- The majority of recurrent SCCHN patients acquire resistance to anti-EGFR therapy
- Preliminary efficacy data suggests that anti-EGFR therapy may confer benefit for SCCS patients, however treatment failure is common [7,8]

Key clinical trials of EGFR-targeted therapies in SCCHN

Drug	Dosage	Indication	N	Response Rate	PFS/TTP (months)	OS (months)
Cetuximab[9]	400 mg/m2 Loading 250 mg/m2 Maintenance	Second-line	103	13%	2.3	5.8
Erlotinib[10]	150 mg/day	First and Second-line	115	4%	2.2	6.0
Gefitinib[11]	500 mg/day	First and Second-line	52	11%	3.4	8.1
Lapatinib[12]	1500 mg/day	First and Second-line	45	0%	1.7	9.5
Afatinib[13]	40 mg/day	Second-line	322	10%	2.6	6.8
Dacomitinib[14]	45 mg/day	First-line	69	13%	2.8	8.0

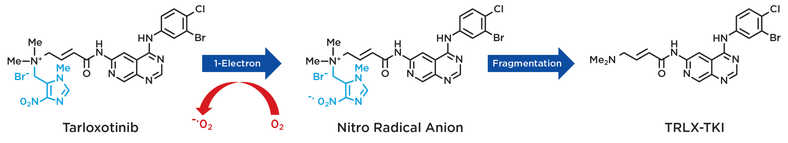
Hypoxia is a therapeutic target for SCCHN

- Most SCCHN tumors contain severely hypoxic regions [15]
- Hypoxia is an independent adverse prognostic factor for SCCHN [16,17]
- Hypoxia promotes the overexpression of EGFR and its cognate ligand TGF-α [18,19]

Tarloxotinib bromide

- Tarloxotinib is a hypoxia-activated prodrug (HAP) that is reduced to a nitro radical anion that acts as a direct 'oxygen sensor' releasing an irreversible EGFR/HER2 inhibitor (TRLX-TKI) under hypoxic conditions
- Attachment of a hypoxia trigger to tarloxotinib significantly reduces the potency of the prodrug, allowing for administration at higher relative concentrations than the cognate TKI (TRLX-TKI)
- Tarloxotinib may overcome resistance and increase the therapeutic ratio over conventional anti-EGFR therapy by inhibiting EGFR with greater dose-intensity in hypoxic tumors

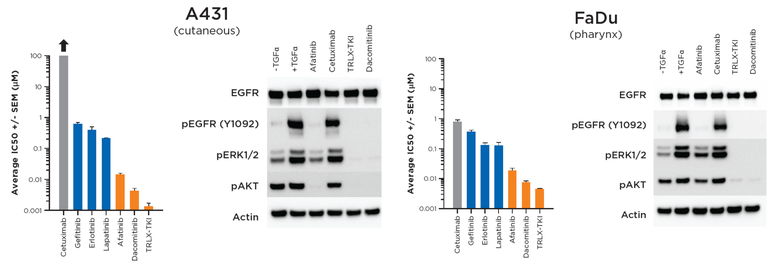
Schematic of tarloxotinib conversion to its irreversible EGFR/HER2 inhibitor



Translational Studies

TRLX-TKI activity and silencing of EGFR signaling in vitro

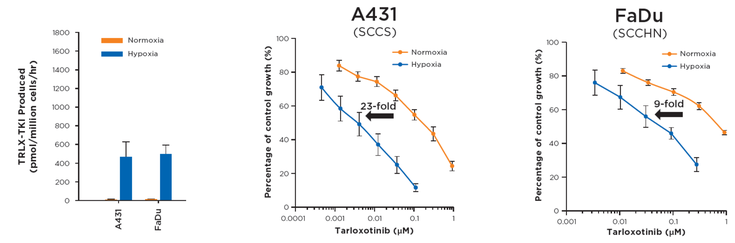
- TRLX-TKI showed greater in vitro activity compared with various EGFR-targeted agents across multiple SCCHN and SCCS cell lines, being up to 5–fold more potent than dacomitinib and up to 11–fold more potent than afatinib on a molar basis



Anti-proliferative assay: Cell lines were exposed to various EGFR-targeted agents under aerobic conditions for five days. Anti-proliferative activity is reported by class of anti-EGFR therapy; monoclonal antibodies (grey), reversible TKIs (blue) and irreversible TKIs (orange). IC50, 50% inhibitory concentration. **Western blot**: Cell lines were exposed to 300 nM of afatinib, cetuximab, TRLX-TKI and dacomitinib for 1 hour with TGF-α (50 ng/mL) induction after 45 min.

Tarloxotinib is a hypoxia-activated prodrug (HAP) in vitro

- Tarloxotinib undergoes hypoxia-selective metabolism to release TRLX-TKI
- Tarloxotinib displays hypoxia-selective anti-proliferative activity

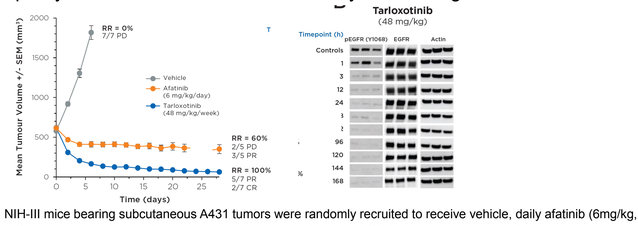


Cellular Metabolism: 5x10^5 cells were exposed to 10 μM tarloxotinib for 90 minutes under aerobic or hypoxic conditions (<1 ppm O_2). Formation of TRLX-TKI was measured by mass spectrometry with reference to a D6 internal standard.

Hypoxic-Cytotoxicity Ratios (HCRs): Cell lines were exposed to tarloxotinib or TRLX-TKI under aerobic conditions for 24 hours with 96 hours of drug-free proliferation, or under hypoxic conditions (<1 ppm O_2) for 4 hours with 20 hours aerobic recovery followed by 96 hours of drug-free proliferation.

Tarloxotinib is efficacious in vivo—SCCS xenograft model

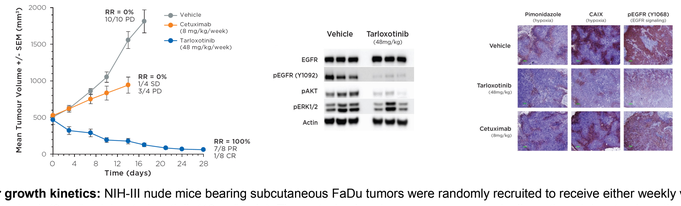
- Tarloxotinib produced 100% tumor regressions in the A431 SCCS xenograft model
- EGFR autophosphorylation remained silenced for 7 day after a single dose



Tumor growth kinetics: NIH-III mice bearing subcutaneous A431 tumors were randomly recruited to receive vehicle, daily afatinib (6mg/kg, po) or weekly tarloxotinib (48mg/kg, ip). Western blot: NIH-III mice bearing subcutaneous A431 tumors were treated with a single dose of tarloxotinib (48mg/kg) and tumors were harvested at various time points after dosing.

Tarloxotinib is efficacious in vivo—SCCHN xenograft model

- Tarloxotinib treatment resulted in 100% FaDu tumor regressions
- Phosphorylation of EGFR and AKT was decreased after tarloxotinib
- Tumor hypoxia was reduced after a single dose of tarloxotinib



Tumor growth kinetics: NIH-III nude mice bearing subcutaneous FaDu tumors were randomly recruited to receive either weekly vehicle (ip), cetuximab (8mg/kg, ip) or tarloxotinib (48mg/kg, ip). The cetuximab-only arm was prematurely discontinued due to ulceration. **Western blot**: tumor-bearing mice were treated with a single dose (as above) and tumor harvested 24 hr later. **Immunohistochemistry**: Tumor bearing mice were treated with a single dose (as above), pimonidazole was administered after 23 hr and tumors harvested at 24 hr

Phase 1 Trial *(completed)*

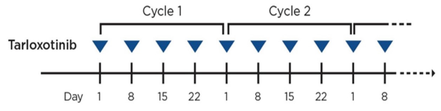
Safety and Pharmacokinetics (PK) - NCT01631279

- 27 patients with advanced solid tumors treated with weekly 1-hour administration
- Dose-escalation: 10—200 mg/m2
- Rash (all were grade 1 or 2), QT prolongation, and nausea were the most common adverse events
- The maximum tolerated dose (MTD) of tarloxotinib was established at 150 mg/m^2/ week (1-hour infusion)
- Dose-limiting toxicities (DLTs) were facial pain (grade 3) and QT prolongation (grade 4) reported in two patients dosed at 200 mg/m^2

Phase 2 Trial *(in progress)*

Study Design - NCT02449681

- Multi-center, open-label
- Population: Confirmed recurrent or metastatic squamous cell head and neck or skin
  - N = 38 to 60 patients
  - Tarloxotinib dose:150 mg/m^2 (1-hour infusion)
- Three parallel disease-specific groups
  - P16-negative SCCHN
  - P16-positive oropharyngeal SCCHN
  - Cutaneous SCC
- Two-stage design (for each disease-specific group)
  - ≥ 1/10 responses to enter second stage (up to 19 more patients)
  - ≥ 4/29 responses to conclude active after second stage



Study Endpoints

Primary objective

- Overall response rate (RECIST v1.1)

Secondary objectives

- Safety and tolerability
- Time to response, PFS, duration of response, disease control rate, time to progression, OS

Exploratory objectives

- [^{18}F]HX4 PET scan at baseline
- Pharmacokinetics
- Tumor tissue at baseline and Day 8

Inclusion Criteria

- Age ≥ 18 years; ECOG Performance Status 0-2
- Histologically or cytologically confirmed squamous cell carcinoma of the head and neck or skin
- For patients with oropharyngeal cancer, p16 status is known or can be determined
- Recurrent disease determined to be incurable by surgery or radiotherapy
- Progression on one or more lines of chemotherapy or other therapy for recurrent or metastatic SCCHN (e.g. cetuximab or immune checkpoint therapy is acceptable) or within 6 months of definitive chemoradiation or bioradiation. Previous systemic therapy is not required for SCCS
- Measurable disease according to RECIST 1.1

References: (1) Cancer Res 1993; **53**: 3579–3584; (2) Cancer 1996; **78**: 1284–1292; (3) J Natl Cancer Inst 1998; **90**: 824–832; (4) Cancer Res 2002; **62**: 7350–7356; (5) Anticancer Res 2005; **25**: 1205–1210; (6) Ann Oncol 2007; **18**: 761-767; (8) Ann Oncol 2014; **25**: 2047–2052; (9) J Clin Oncol 2007; **25**: 2171-2177; (10) J Clin Oncol 2004; **22**: 77–85; (11) J Clin Oncol 2003; **21**: 1980-1987; (12) Clin Cancer Res 2012; **18**: 2336 –2343; (13) Lancet Oncol 2015; **16**: 583–594; (14) Ann Oncol 2013; **24**: 761–769; (15) Cancer Res; 2011; **71**: 5923-5931; (16) Int J Radiat Oncol Biol Phys 2012; **84**:807-814; (17) Strahlenther Onkol 2003; **179**: 521–526; (18) Proc Natl Acad Sci USA 2007; **104**: 13092–13097; (19) Carcinogenesis 2010; **31**: 1202– 1210; (20) AACR-NCI-EORTC Conference; 2015: abstract A66. ***Disclosure:*** Tarloxotinib is currently under clinical investigation and has not been approved by any regulatory authority. ***Status:*** May 2016.