Evaluation of Drug-Drug Interactions (DDI) Between Tucatinib and Capecitabine in Patients With Advanced HER2+ Metastatic Breast Cancer From a Phase 1b Study

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Background

- Tucatinib (ONT-380) is a HER2 selective small molecule tyrosine kinase inhibitor with nanomolar potency
  - HER2 IC_{50}: 8 nM; EGFR IC_{50}: >10,000 nM using a cellular signalling assay
- Tucatinib is highly active in murine tumor models of HER2+ disease
  - Synergy with trastuzumab and chemotherapy
  - Improved survival compared to lapatinib and neratinib in a preclinical model of CNS disease
- Tucatinib is being evaluated in a Phase 1b study in combination with capecitabine (C; Xeloda®) and trastuzumab (T; Herceptin®) in metastatic breast cancer (MBC) patients with or without CNS metastases (Study ONT-380-005), based on:
  - Potential for increased anti-tumor activity with dual blockade of HER2
  - Improved preclinical antitumor activity of tucatinib with chemotherapy
  - Potential for activity against CNS metastases
- Parallel 3+3 dose escalation of tucatinib in combination with either C alone or T alone, followed by evaluation in combination with both C and T
  - Expansion cohorts at MTD/Phase 2 recommended dose (P2RD) in patients with and without response-assessable CNS metastases

Introduction

- Capecitabine is a chemotherapeutic prodrug used to treat metastatic breast and colorectal cancers that is bio-transformed into the active antimetabolite 5-fluorouracil (5-FU)
- Purpose of this work was to evaluate the potential effect of tucatinib and its metabolite ONT-993 on the catabolism and metabolism of capecitabine using *in vitro* enzymatic assays and clinical pharmacokinetic analysis

Tucatinib metabolism and CYP450 interaction:

- Metabolized by cytochrome P450 2C8 mainly and by 3A4 to a lesser extent
- ONT-993, a hydroxylated product of tucatinib, has been identified as a major metabolite
- Inhibits CYP 2C8, 2C9 and 3A4 moderately *in vitro* (~9-17 µM)

CYP450	IC_{50} (µM)
1A2	> 25 µM
2C19	> 25 µM
2C8	8.8
2C9	10.5
2D6	21.1
3A4	13.0 / 16.9

Table 1. *In vitro* inhibition of CYP450 by tucatinib

Capecitabine metabolism:

- Pro-drug, converted to 5-FU (the active antimetabolite) by liver carboxylesterases in a series of sequential activation steps
- 5-FU is subsequently inactivated by dihydropyrimidine dehydrogenase (DPD), which is polymorphically expressed
- Not metabolized by cytochrome P450 and is not a CYP450 inhibitor (IC_{50} > 20 µM). Since tucatinib is metabolized mainly by CYP450 enzymes, there is little risk of capecitabine having an effect on the metabolism of tucatinib

Capecitabine Metabolic Pathway in Humans

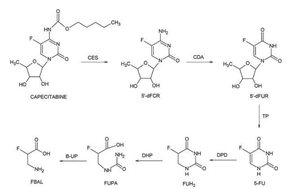
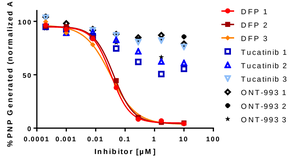


Figure 1. Capecitabine metabolic conversion to 5-FU active anti-metabolite and its metabolism. Enzymes evaluated in this study were carboxyesterase (CES), cytidine deaminase (CDA), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD). 5'-dFCR is 5'-deoxy-5-fluorocytidine, 5'-dFUR is 5'-deoxy-5-fluorouridine, 5-FU is 5-fluorouracil, FUH2 is dihydro-5-fluorouracil, FUPA is 5'-fluoro-ureido-propionic acid, FBAL is α-fluoro-β-alanine or α-fluoro-propionic acid

Tucatinib and ONT-993 Do Not Inhibit Carboxylesterase

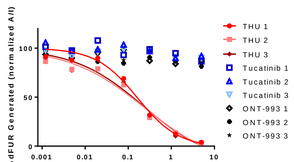
Compound Tested	Mean IC_{50} (µM)	Mean % Inhibition @ 2µM, (rh/c)
DFP	0.0353	95 / 81
Tucatinib	nd	35 / 27
ONT-993	nd	20 / 17



Table/Figure 2. Effect on carboxylesterase (CES) activity by tucatinib, ONT-993 and diisopropyl fluorophosphate (DFP, a positive control inhibitor) using recombinant enzyme (rh) or liver cytosol (c). Specific enzymes and substrate para-nitrophenyl acetate (the metabolite monitored is para-nitrophenol or PNP) were incubated in an appropriate buffer and the reaction was monitored in the absence or presence of test compounds. All compounds were tested in triplicate. The substrate concentration in the assay was chosen near the published K_m. In situations where the K_m was not available, enzymatic kinetic studies were conducted to determine the K_m experimentally. No remarkable inhibition of CES was observed with either tucatinib or ONT-993. nd = an IC_{50} value could not be determined under these test conditions

Tucatinib and ONT-993 Do Not Inhibit Cytidine Deaminase

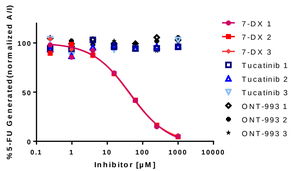
Compound Tested	Mean IC_{50} (µM)	Mean % Inhibition @ 2µM
THU	0.207	91
Tucatinib	nd	8
ONT-993	nd	13



Table/Figure 3. Effect on cytidine deaminase (CDA) activity by tucatinib, ONT-993 and tetrahydrouridine (THU, positive control inhibitor). CDA (in liver cytosol) and a specific substrate 5'-dFCR (the metabolite 5'dFUR was monitored) were incubated in an appropriate buffer and the reaction was monitored in the absence or presence of test compounds. All compounds were tested in triplicate. No remarkable inhibition of CDA was observed with either tucatinib or ONT-993

Tucatinib and ONT-993 Do Not Inhibit Thymidine Phosphorylase

Compound Tested	Mean IC_{50} (µM)	Mean % Inhibition @ 2µM
7-DX	44.0	8
Tucatinib	nd	5
ONT-993	nd	2

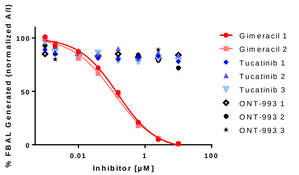


Table/Figure 4. Effect on thymidine phosphorylase (TP) activity by tucatinib, ONT-993 and 7-deazaxanthine (7-DX, positive control inhibitor). TP (in liver cytosol) and specific substrate 5'-dFUR (the metabolite 5-FU was monitored) were incubated in an appropriate buffer and the reaction was monitored in the absence or presence of test compounds. All compounds were tested in triplicate. No remarkable inhibition of TP was observed with either tucatinib or ONT-993

CASCADIAN THERAPEUTICS

Tucatinib and ONT-993 Do Not Inhibit Dihydropyrimidine Dehydrogenase

Compound Tested	Mean IC_{50} (µM)	Mean % Inhibition @ 2µM
Gimeracil	0.136	92
Tucatinib	nd	18
ONT-993	nd	19



Table/Figure 5. Effect on dihydropyrimidine phosphorylase (DPD) activity by tucatinib, ONT-993 and Gimeracil (positive control inhibitor). DPD (in liver cytosol) and specific substrate 5'-dFUR (the metabolite FBAL was monitored) were incubated in an appropriate buffer and the reaction was monitored in the absence or presence of test compounds. The positive control was tested in duplicate and tucatinib and ONT-993 were tested in triplicate. No remarkable inhibition of DPD was observed with either tucatinib or ONT-993

ONT-380-005 Clinical Study Overview

- 3+3 dose escalation of tucatinib tablets in combination with capecitabine or trastuzumab alone, followed by evaluation of tucatinib in combination with both capecitabine and trastuzumab
  - Tucatinib (300 mg and 350 mg) PO BID
  - Capecitabine 1000 mg/m^2 BID Days 1-14
  - Trastuzumab 8 mg/kg IV Cycle 1 Day 1, then 6 mg/kg QDx21 days for Cycles 2-6.
- Expansion cohorts in patients with CNS metastases
- Patient Population: HER2+ breast cancer with progression after prior therapy with trastuzumab and T-DM1 for metastatic disease
- Pharmacokinetics Objectives:
  - Tucatinib PK schedule: Cycle 1 Day 14 and 21 Cycles 2-6 Day 1
  - Capecitabine PK schedule: Cycle 1 Day 14 & 21
  - PK Analyses: tucatinib, ONT-993 metabolite, capecitabine, 5'-dFCR, 5'-dFUR, 5-FU, and FBAL

Tucatinib Plasma Concentration Profiles Are Dose Dependent

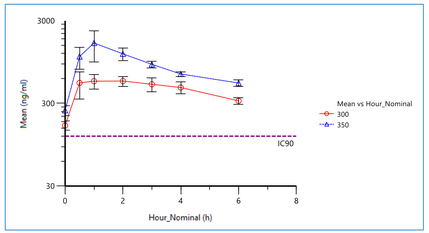


Figure 6. Cycle 1 Day 14 plasma concentration profiles of tucatinib from the tucatinib+C cohort. Tucatinib was given orally at doses of 300 mg BID (red) or 350 mg BID (blue) in combination with capecitabine. Exposure seems proportional with dose. Reference line refers to the concentration of tucatinib that inhibited 90% of HER2 phosphorylation in a cell based assay

Capecitabine Has No Effect on Tucatinib Plasma Concentration Profile

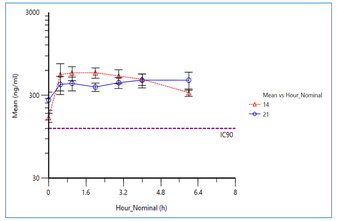


Figure 7. Tucatinib plasma concentration profiles in combination with capecitabine. Tucatinib was given orally at doses of 300 mg BID in combination with capecitabine on day 14 (red) and without capecitabine on day 21 (blue). There was no difference in the tucatinib plasma concentration profiles with or without capecitabine

Capecitabine and Metabolites PK Profiles

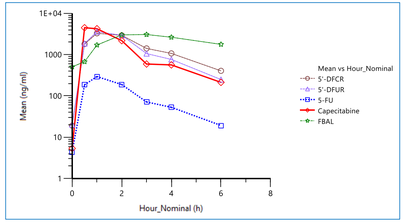


Figure 8. Plasma concentration profiles of capecitabine and its metabolites. Capecitabine was given orally at 1000 mg/m^2 in combination with tucatinib for 14 days in a 21 day cycle. PK assessments were done on day 14 for capecitabine and the metabolites 5'-dFCR, 5'-dFUR, 5-FU & FBAL

Pharmacokinetic Parameters of Tucatinib

Tucatinib Dose	Treatment	PK Day (Cycle 1)	PK Parameter	N	Mean	SE	% CV	Geometric Mean	% CV Geometric Mean
300 mg BID	tucatinib + C	14	T_{max}	6	1.8	0.6	78	1.4	111
			C_{max}	6	781	145	46	727	41
			AUC_{last}	6	2793	370	32	2673	34
			AUC_{TAU}	6	4055	445	27	3930	28
		21	T_{max}	6	3.9	1.1	66	2.8	150
			C_{max}	6	581	101	43	539	45
			AUC_{last}	6	2514	405	39	2372	38
			AUC_{TAU}	3	3904	864	38	3674	47
	tucatinib + C + T	14	T_{max}	11	2.6	0.3	37	2.4	42
			C_{max}	11	1080	139	43	1006	40
			C_{min}	11	275	48	58	233	68
			AUC_{last}	11	4367	556	42	4070	40
			AUC_{TAU}	11	5785	631	36	5484	35
350 mg BID	tucatinib + C	14	T_{max}	3	1.2	0.4	65	1	79
			C_{max}	3	1696	610	62	1483	71
			AUC_{last}	3	5403	875	28	5244	32
			AUC_{TAU}	3	7396	1056	25	7228	27

Table 6. Tucatinib pharmacokinetic (PK) parameters. Tucatinib was given orally at doses of 300 and 350 mg BID in combination with capecitabine (tucatinib+C) or with capecitabine and trastuzumab (tucatinib+C+T). PK assessments were done on day 14 and day 21 of cycle 1

Tucatinib Pharmacokinetics Summary

- Tucatinib plasma exposure seems to increase with dose
- There is no difference in the tucatinib plasma concentration profiles with or without capecitabine
- Tucatinib levels were similar in both combinations with capecitabine and with capecitabine/trastuzumab

Pharmacokinetic Parameters of Capecitabine

Analyte	Half-life (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	AUC_{inf} (h*ng/mL)	Literature AUC*
Capecitabine	0.7	1.5	6144	8862	9124	4384-5777
5'-dFCR	3.9	1.8	4341	9113	12065	5208-11235
5'-dFUR	0.9	2.0	4746	9008	9926	9323-12800
5-FU	1.6	1.6	348	661	737	369-556
FBAL	2.7	2.8	3845	13618	18588	18320-40717

** (Reigner et al., Clin Pharmacokinet (2001) 40(2):85-104; values normalized to 1000 mg/m^2 capecitabine)*

Table 7. Capecitabine PK parameters. Capecitabine was given orally at 1000 mg/m^2 in combination with tucatinib for 14 days in a 21 day cycle. PK assessments were done on day 14 for capecitabine and the metabolites 5'-dFCR, 5'-dFUR, 5-FU & FBAL. The PK parameters for capecitabine and its metabolites appears to be consistent with reported literature values

Summary and Conclusion

In Vitro DDI Results:

- Tucatinib and ONT-993 did not remarkably affect the activity of major enzymes involved in the metabolism of capecitabine *in vitro*
- Capecitabine did not have any measurable effect on the activity of CYP450 mediated metabolism of tucatinib *in vitro*
- *In vitro* results suggest minimal risk of significant drug-drug interaction for the combination of tucatinib with capecitabine *in vivo*

Clinical Pharmacokinetic Results:

- Capecitabine did not have any measurable effect on tucatinib plasma concentration profile or PK parameter values
- Capecitabine and its metabolites PK profiles in the presence of tucatinib appears to be consistent with reported literature values of capecitabine dosed alone

CONCLUSION:

- Combination of tucatinib with capecitabine or with capecitabine/trastuzumab did not affect the pharmacokinetics of tucatinib or capecitabine
- The results suggest no risk of drug-drug interaction between tucatinib and capecitabine
- The safety data from this study supports the use of the triplet combination (tucatinib+C+T) in the pivotal trial HER2CLIMB (NTC02614794)

This poster can be downloaded at http://www.cascadianrx.com