Newsletter

This resume of the results from the phase 1 study with Foxy-5 is based on clinical and laboratory data from the study, and these data have now been locked into the database. The full report will not be published at this stage, due to pending patent issues and competitor matters.

Phase 1 Dose Escalating Study to Evaluate the Safety, Tolerability, Pharmacokinetic and Pharmacodynamic Profiles of Foxy-5 in Patients with Metastatic Breast, Colon or Prostate Cancer.

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Primary Objective:

- To evaluate the safety and tolerability of treatment with Foxy-5

Secondary Objectives:

- To determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of Foxy-5 in patients with metastatic breast, colon or prostate cancer
- To characterize the pharmacokinetic (PK) profile of Foxy-5
- To characterize the pharmacodynamic (PD) profile of Foxy-5
- To characterize the profile for the biomarkers NGAL, 15-PDGH and PGE2 in tumor tissue/blood, and estimate the number of circulating tumor cells before and after treatment with Foxy-5
- To characterize the mRNA expression (genome wide), protein expression of Wnt-5a and NGAL, and perform hematoxylin-eosin (HE) staining of voluntary tumor biopsies obtained prior to Day 1 and after 2 weeks treatment on Day 12.

OBJECTIVES

This study was an open-label phase 1 dose-escalating study to evaluate the safety, tolerability, dose limiting toxicities (DLT) and maximum tolerated dose of Foxy-5 in patients with metastatic breast, colon or prostate cancer for which no approved standard treatment was available. Additional secondary objectives were to characterize the pharmacokinetic and pharmacodynamic profiles of Foxy-5 as well as characterize the profile of NGAL, 15-PGDH and PGE2 in tumor tissue/blood, and estimate the number of circulating tumor cells before and after treatment with Foxy-5.

DESIGN

The MTD (maximum tolerable dose) as determined using a standard 3+3 dose escalation cohort design, where 3 to 6 patients were treated at each dose level, and the escalation to the next dose level was permitted depending on the outcomes of the previous dose levels. Escalation to the next dose level was dependent on approval of an independent safety data monitoring committee (SDMC) that evaluated all relevant safety data for each dose cohort once the last patient in the cohort had completed one cycle of treatment. Cohort expansion was required if 1 dose-limiting toxicity (DLT) was reported. The dose escalation should be stopped if 2 patients experienced a DLT at a given dosage level, and the preceding cohort would then be expanded to 6 patients, assuming that no DLTs were reported, this would be classified as the Recommended Dose (RD). Patients received one intravenous slow infusion of Foxy-5 three times weekly on Mondays, Wednesdays and Fridays for three weeks constituting one cycle. During this cycle the pharmacokinetics were investigated. The next cycle followed immediately after, with no interruption between cycles. Patients could continue to receive Foxy-5 treatment at the investigator's discretion until disease progression or unacceptable toxicity was encountered. CT scans were performed before treatment started and then every 8 weeks until disease progression or withdrawal and at the end of treatment (EoT). The patient's tumor response was then assessed according to RECIST criteria (version 1.1). Following disease progression or withdrawal, patients were followed-up for 30 days after end of treatment.

RECRUITMENT OF PATIENTS

A total of 31 patients were included in the study of which 27 were evaluable, meaning that they completed at least 9 treatments (3 weeks) with Foxy-5. For the higher doses of Foxy-5 we only included patients with no or low Wnt5a protein expression in their primary tumors.

TREATMENT

Patients received one intravenous slow infusion of Foxy-5 three times weekly for 3 weeks (1 cycle). Foxy-5 treatment continued at the Investigator's discretion. A total number of 8 dose levels were tested (0.013mg/kg-1.3 mg/kg) (see Figure 1).

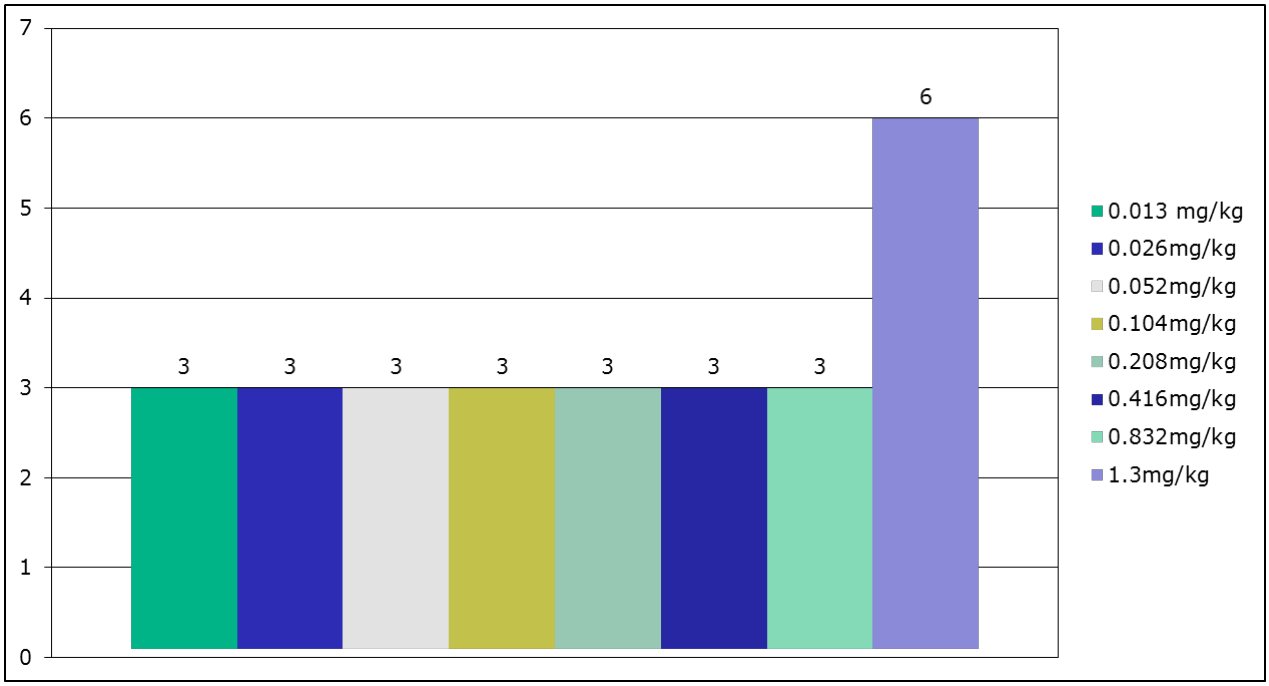

Figure 1 shows the number of patients who completed each dose level of Foxy-5

As can be seen in Figure 2, many of the patients received additional infusions of Foxy-5. The per protocol number of Foxy-5 infusions were 9 infusions (3 infusions per week for 3 weeks) but the mean number of infusions of Foxy-5 turned out to be 23 with three patients receiving 50 or more infusions.

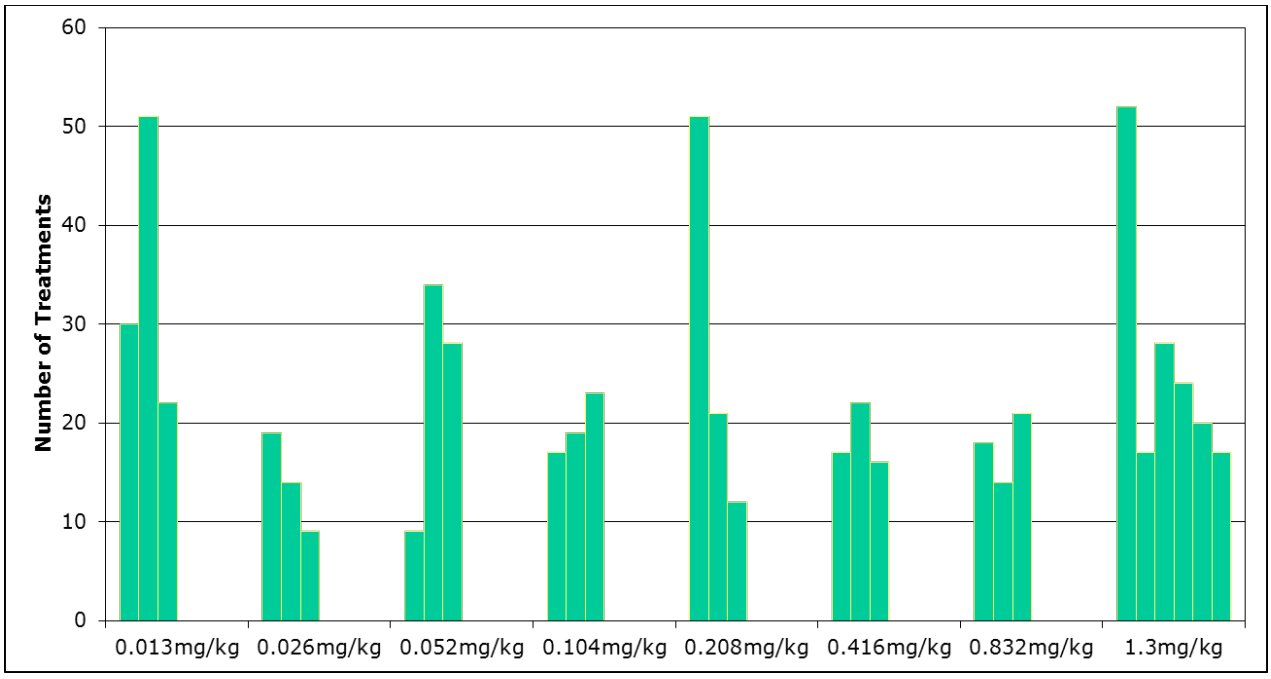

Figure 2 shows the number of infusions with Foxy-5

PRIMARY OBJECTIVE

Toxicity from Foxy-5 treatment was assessed by NCI Common Toxicity Criteria for Adverse Events (CTCAE). The study showed that Foxy-5 was a safe and well tolerated drug at all dose levels. We were particularly interested in observing signs of toxicities related to white blood cells, liver function, renal function and the neurological system as these tissues/cells are most often affected by anti-cancer therapy.

SECONDARY OBJECTIVES

Since no dose limiting toxicities were observed a maximum tolerated dose (MTD) could not be defined. However, some serious adverse events (SAE's) were observed and the most prevalent of these were general disorders and administration site conditions (pyrexia was the most common). Four SAEs were classified as unlikely related to treatment (chest pain, pyrexia and hypertension), and all others were rated as unrelated to treatment, and none were considered to be dose limiting.

As part of the secondary objectives the pharmacokinetics and pharmacodynamics of Foxy-5 were also studied.

Pharmacokinetics: Foxy-5 could be detected in the blood of patients given the highest levels of Foxy-5 already within the first 5 minutes after infusion. There was a clear dose/response relationship (the higher dose the more Foxy-5 in the blood) for all dose levels. The Foxy-5 concentration was below the lower limit of detection 2 days after first infusion in cohorts 1 and 2 and undetectable in cohort 3 by the start of the second week of treatment. In cohorts 4 and onwards Foxy-5 could be detected in the blood throughout the treatment cycle (3 weeks, 9 infusions).

Pharmacodynamics: According to the protocol, measurements before and during Foxy-5 treatment of the following parameters were used to estimate Foxy-5 pharmacodynamics:

1. Circulating tumor cells before and during Foxy-5 treatment
2. NGAL plasma content before and during Foxy-5 treatment
3. 15-PGDH and PGE2 tumor/blood content before and during Foxy-5 treatment
4. mRNA expression in tumors before and during Foxy-5 treatment
5. Wnt-5a tumor cell content before and during Foxy-5 treatment
6. CT scans before, during and after Foxy-5 treatment

Circulating tumor cells before and during Foxy-5 treatment: Measurements of circulating tumor cells in the blood were obtained from dose level 5 and up. As seen from Figure 3, not all the patients had detectable cancer cells in the blood even though they had metastatic cancer. It is noteworthy that in 7 of the patients, the number of circulating cancer cells increased from day 1 to day 19 of Foxy-5 treatment while it remained stable in 5 of the patients. The patient with stable disease (see below) also had stable numbers of circulating cancer cells. It must be emphasized that the results from counting circulating cancer cells are difficult to evaluate, since it has been shown in mouse experiments, that Foxy-5 impairs at least two different processes, release of tumor cells from tumor tissues (decreasing the number of CTC's) and the passage of cancer cells out of the blood stream (increasing the number of CTC's). Moreover, CTC numbers were analyzed in a machine that is based on recognition of specific epithelial markers on cells in the blood for counting circulating cancer cells and it has now been shown that this method may not be sufficient. In particular, this method does not detect cancer cells that have undergone what is called "epithelial-

mesenchymal transition" and since this transition is considered as a main step in the metastatic process, we might have missed detection of a crucial fraction of circulating tumor cells. WntResearch has now entered an agreement with Professor Klaus Pantel from Hamburg who is considered one of the world experts in measuring circulating cancer cells, and Professor Pantel will lead and perform future measurements of circulating cancer cells, in our clinical trials, in his laboratory.

Cohort	Patient No.	Diagnosis	Wnt-5a status	Day 1	Day 12	Day 19
5	01-0016	C. Prostate	loss or reduced	69 & 70	49 & 56	63 & 66
	02-0001	C. Bladder	normal level	94 & 92	276 & 302	267 & 215
6	02-0002	C. Colon	normal level	9 & 13	12 & 10	29 & 33
	01-0017	C. Colon	normal level	2 & 1	2 & 2	2 & 2
	01-0018	C. Prostate	loss or reduced	826	**	**
7	01-0019	C. Breast	loss or reduced	98 & 100	161 & 145	338
	02-0004	C. Colon	loss or reduced	10 & 11	**	10 & 13
	02-0005	C. Colon	loss or reduced	1 & 4	1 & 2	6 & 7
8	01-0020	C. Prostate	loss or reduced	1 & 1	2 & 1	1 & 4
	01-0021	C. Prostate	loss or reduced	18 & 12	15 & 26	36 & 36
	02-0006	C. Breast	loss or reduced	0	0 & 0	0 & 0
	02-0007	C. Colon	loss or reduced	5 & 3	5 & 6	**
	01-0023	C. Prostate	loss or reduced	2	2 & 3	5 & 7
	01-0024	C. Colon	loss or reduced	1 & 1	5 & 6	8 & 14
	01-0025	C. Prostate	loss or reduced	87 & 106	91 & 94	83 & 93
	01-0026	C. Colon	loss or reduced	0 & 0	0 & 0	0 & 0

Figure3: Number of CTC (circulating cancer cells) at day 1 before treatment, day 12 and day 19 after Foxy-5 treatment

NGAL plasma content before and during Foxy-5 treatment: There exists indirect support for using plasma levels of the protein NGAL as a potential biomarker for Foxy-5-induced biological effects. NGAL in blood obtained from the patients before and during Foxy-5 treatment was measured. The data below (Figure 4) does not reveal a clear-cut indication that plasma NGAL levels can be used as a biomarker for detecting a biological effect of Foxy-5. It is possible that analysis of NGAL levels in the urine would constitute a more sensitive approach for detecting Foxy-5-induced changes in NGAL levels.

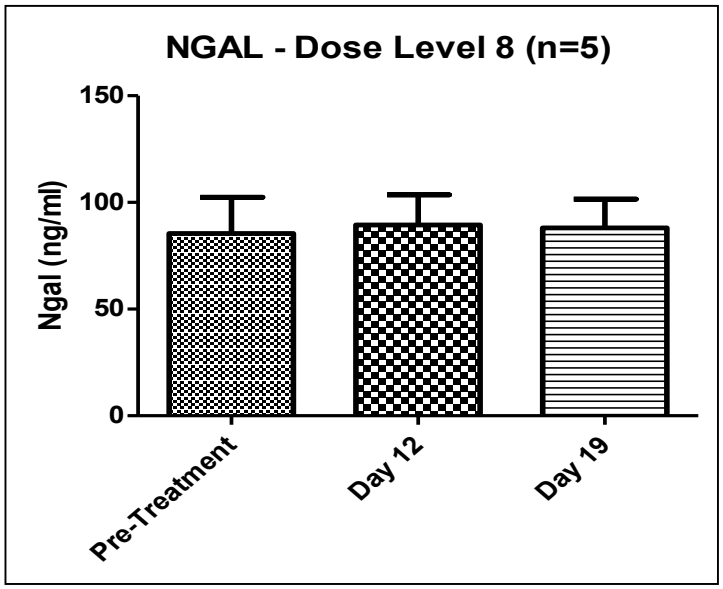

Figure 4: NGAL protein levels in the blood pre-treatment and after 12 and 19 day of Foxy-5 treatment

15-PGDH and PGE2 tumor/blood content before and during Foxy-5 treatment: These two molecules should be measured in the tumor biopsies/blood obtained before and during Foxy-5 treatment. Due to incomplete laboratory methods theses analyses were not performed. We therefore prioritized the mRNA expression studies of the tumor biopsies.

mRNA expression in tumors before and during Foxy-5 treatment: The voluntary sampling of biopsies from tumors before and during Foxy-5 treatment started later in the study which resulted in only three patients consenting to collection of these biopsies. One of these patient biopsies appeared not to fulfill the quality criteria for mRNA expression analysis, leaving two patients for the analyses. However, in the biopsies from these two patients we observed clear changes in gene expression between pre-treatment and treatment biopsies. Some of the changed gene products are known to be involved in metastatic spread of cancer, while others have not previously been associated with spreading of cancer. Due to patent and competition issues, the names of the genes are not disclosed here. In the phase 1b study, one of the inclusion criteria for the patients is that they should have tumor lesions accessible for biopsies which will allow us to further validate these findings from the phase 1 study.

Wnt-5a tumor cell content before and during Foxy-5 treatment: This was inconclusive, due to methodological difficulties. The experiences gained are used for the coming studies.

CT scans before and during Foxy-5 treatment: As measured by CT scans, one of three patients in dose level 5 and one of six patients in dose level 8 obtained a confirmed stable disease condition following treatment with Foxy-5. Confirmed stable disease means that the patient had stable disease at two independent CT scans (week 8 and week 16 after treatment). The patient in dose level 8 who obtained long lasting stable disease was a 78 year old man with prostate cancer. His tumor was negative/low in Wnt5a. He also had stable circulating cancer cell numbers. Prior preclinical tumor experiments have shown that Foxy-5 does not induce tumor regression (tumors become smaller) but it inhibits the spreading of cancer. Regression of the tumors in the Foxy-5 treated patients were thus not expected. Whether the observed stable diseases are a result of the Foxy-5 treatment or something else cannot be determined by our study since 1) the number of patients in each treatment group was very small and 2) we did not have any control group in the study. In the upcoming phase 2 clinical study, we will randomize the patients to either receive standard treatment or standard treatment plus Foxy-5 which will allow us to perform a comparison with a control group.

DISCUSSION AND CONCLUSIONS

The planed clinical phase 1 study, with 3 patients at dose levels 1-7 and 6 patients at dose level 8, has been finalized. The main finding is that Foxy-5 is safe and that no dose limiting toxicity was found at the dose levels applied. This finding is in accordance with all rat and dog toxicity studies. This observation is extremely important as Foxy-5 is meant to be combined with cytotoxic drugs, e.g. chemotherapy, thereby opposing cancer progression by different means. If Foxy-5 had shown toxic effects on the patients, it could have been a problem to combine Foxy-5 with chemotherapy in future clinical trials, due to their additive toxic effects.

Since we did not find any dose limiting toxicity we could not determine maximum tolerated dose of Foxy-5. Thus, we do need another way to establish the optimal dose of Foxy-5 to be used in the clinical phase 2 studies. We therefore measured a number of biological parameters including the effects on tumor size (CT-scans). The results from measuring circulating tumor cells are encouraging but still there are too few observations (too few patients) and too few circulating tumor cells to draw any firm conclusions. However, based on the results we have decided to intensify the circulating tumor cell measurements including using more sophisticated methods in the phase 1b study. We were able to obtain good quality tumor biopsies before and during treatment from two patients. Of particular note is that in both patients, we observed clear changes in gene expression between samples taken before and during treatment, including some genes that have previously been associated with metastatic spread of cancer. These results must be interpreted with caution and considered as preliminary. Much more emphasize will be placed on analysis of tumor biopsies in the phase 1b study and we will then validate the mRNA findings from the phase 1 study.

In the phase 1 study, we were only allowed to enroll cancer patients who already had metastatic disease. Thus, we could not validate the effects of Foxy-5 on metastatic spread in this study. This will be done in the upcoming phase 2 study where we will enroll cancer patients with newly operated cancer and with no signs of metastases but with a high risk of later development of metastases. As per protocol, we performed CT scans every 8 weeks following treatment initiation. We did observe two patients with a confirmed stable disease as determined by CT scans. Due to the limited number of patients in this study and the lack of an untreated control group, we are not able to make any firm conclusions on this finding. When we perform the phase 2 study, numerous patients will be included and there will be a control group that does not receive Foxy-5 allowing us to validate the present CT scan results.