Long term safety observed with CEQ508: An oral RNAi drug targeting β-catenin of GI polyps

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Abstract

Marina Biotech is developing a proprietary drug discovery and delivery platform called transkingdom RNAi (*tk*RNAi), RNAi-based therapeutics in which non-pathogenic bacteria are engineered to: (1) express short hairpin RNA (shRNA), (2) invade via a specific cell surface receptor and (3) deliver shRNA to the cytoplasm of a targeted cell. CEQ508, an *E. coli* derivative delivered orally, is the first drug candidate utilizing this approach. CEQ508 expresses a shRNA directed against β-catenin, and has been shown to significantly reduce β-catenin mRNA levels *in vitro*. In the colorectal cancer cell line, SW480, we observed 90% suppression of β-catenin mRNA. β-catenin is a key oncogene implicated in Colorectal Cancer (CRC) and Familial Adenomatous Polyposis (FAP). FAP is a hereditary disease in which the formation of hundreds of polyps in the gastrointestinal tract ultimately leads to the development of colon cancer. CEQ508 will be tested in a Phase Ib/IIa clinical trial in FAP patients at Massachusetts General Hospital with first patient dosing expected in the second quarter of 2011. CEQ508 has received orphan drug status for the intended treatment of FAP patients by the FDA's Office of Orphan Products Development.

In preparation for later pivotal clinical trials, we recently completed a 9 month GLP non-human primate toxicology study assessing the safety of daily, oral chronic administration of CEQ508. In this study, 18 animals were dosed with either CEQ508 or two control articles. No CEQ508-related adverse responses were identified in the following study parameters analyzed: clinical observations, body weights and temperatures, serum chemistry, coagulation, hematology, urinalysis, cytokines and gross pathology. Preliminary conclusions identify the No Observed Adverse Effect Level (NOAEL) for long-term daily oral administration of CEQ508 as $1x10^{11}$ colony forming units (cfu)/day. In addition to presenting additional details of long term toxicology study, a clinical update on our Phase Ib/IIa trial will be provided.

*tk*RNAi Technology & Background

Figure 1

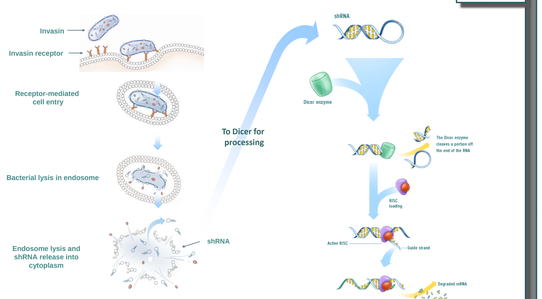


Figure 1: The *tk*RNAi platform involves the modification of bacteria to deliver shRNA to cells of the intestinal tract. A significant advantage of the platform is oral (by mouth) delivery, making this platform extremely patient friendly while harnessing the full potential of the RNAi process. This is accomplished by engineering the bacteria with key attributes as described above.

Figure 2a-d

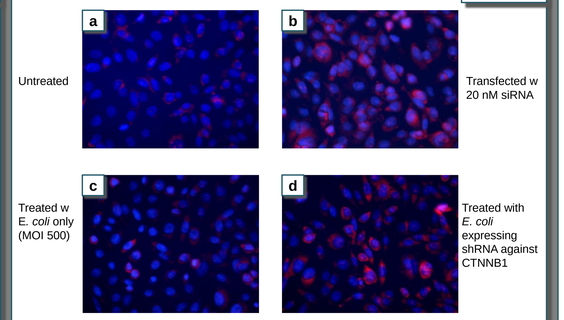


Figure 1a-d: Bacterial mediated *tk*RNAi delivery to epithelial cells (SW480). Therapeutic shRNA (d) is detected in the cytoplasm of epithelial cells by *in situ* hybridization at levels comparable to transfected siRNA (b).

Figure 3

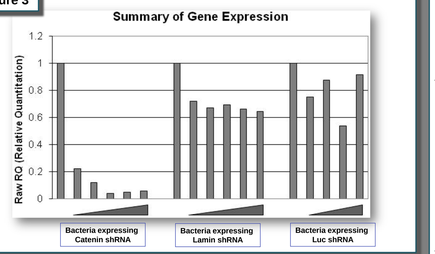


Figure 3: Epithelial cells (SW480) were treated with increasing amounts of CEQ508 and control bacteria (targeting 2 different hairpins-lamin and luciferase) and analyzed for β-catenin expression.

Figure 4

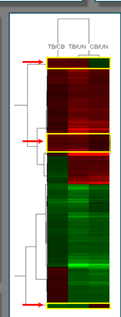


Figure 4: Data derived from epithelial (SW480) cells treated with: β-catenin *tk*RNAi (TB), lamin *tk*RNAi control (CB), and untreated (UN). Down-regulation of β-catenin is associated with *tk*RNAi treatment. Genes known to be upstream regulators of β-catenin are not differentially expressed under any condition. The expression of TLRs themselves does not seem to be dramatically altered at the time point at which these samples were taken. Subsequent analysis in PBMCs treated with *tk*RNAi showed very minimal and transient upregulation of cytokine levels analyzed.

Figure 5

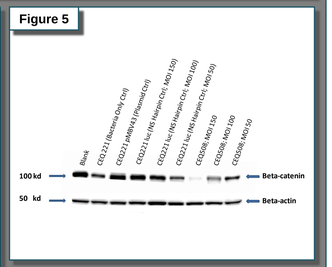


Figure 5: Efficient, dose dependent reduction of β-catenin protein levels *in vitro* (SW480) by *tk*RNAi/bacterial delivery system; compared to bacteria only and irrelevant hairpin control.

APC^{min} mouse model of FAP

- Mutation in APC (adenomatous polyposis coli) gene
- Model for human FAP
- Loss of allele results in activated wnt-TCF4 signaling
- Develop multiple intestinal polyps with bleeding and obstruction (rare carcinoma) progressive with age, death by age 18-20 weeks
- β-catenin acts as a transcription factor and when deregulated due to the APC mutation accumulates in the nucleus leading to hyperproliferation and dysplasia

Figure 6

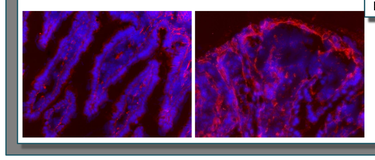


Figure 6: β1-integrin receptors in normal villi (left panel) are basolaterally located, preventing the uptake of the *tk*RNAi bacteria in normal tissue. Receptors reorient to the apical surface in polyposis tissue (right panel) and facilitate the invasin-β1-integrin invasion process.

Figure 7

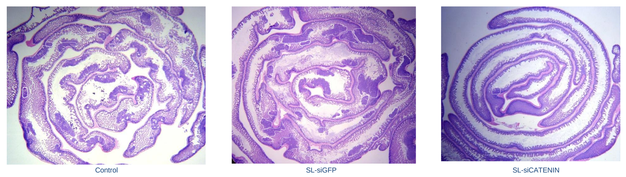


Figure 7: Oral treatment of APC^{min} mice with attenuated *S.typhimurium* encoding for shRNA against mouse β-catenin (SL-siCATENIN) resulted in a significant reduction (prevention) of polyp formation (treatment duration of 6 weeks, starting at 12 weeks of age).

NHP Long-term and Phase IIb-enabling Toxicology Study

Figure 8

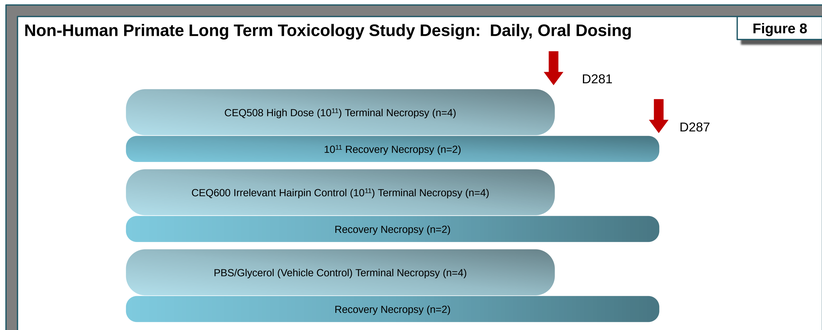


Figure 9a

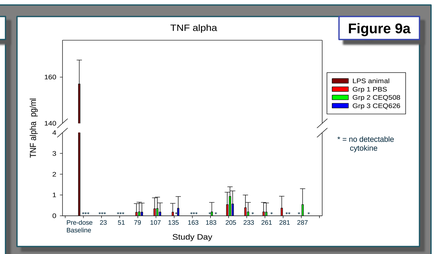


Figure 9a-b: Serum collected pretreatment and monthly throughout the study was analyzed for Th1 cytokine induction. Treatment with CEQ508 did not induce a cytokine response in the panel of pro-inflammatory markers analyzed. Serum collected from a LPS injected NHP was used as a intra-assay positive control.

Figure 10a

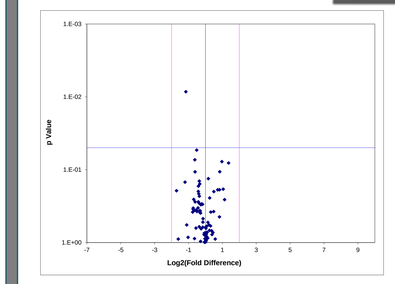


Figure 10b

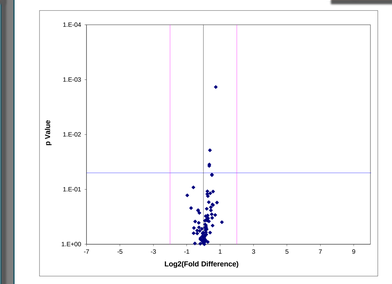


Figure 9b

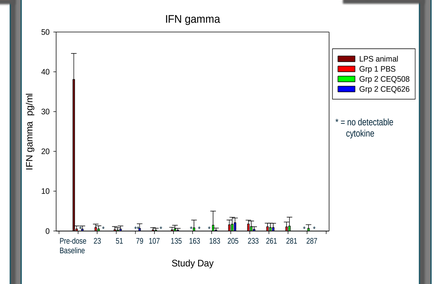


Figure 8:
The study design of the long term toxicology study included 18 non-human primates dosed orally and daily for 9 months with 10^{11} colony forming units (cfu) of CEQ508. This dose level is the highest dose anticipated in the Dose Escalating Phase of our Phase Ib/IIa clinical trial in adult FAP patients.

Figure 10a-b:
Colonic tissue was collected (a) pretreatment (b) and after six months of daily treatment and local cytokine response was analyzed. The gene array analysis monitored the activity of 84 different cytokines, chemokines and related receptors, and compared PBS treated control animals to CEQ508 treated samples. Treatment with CEQ508 did not induce a local cytokine activation.

Safety Summary:
In 280 days of daily oral dosing, no test article-related adverse responses were identified in a variety of parameters, including: clinical observations, body weights and temperatures, serum chemistry, coagulation, hematology, serum and local cytokines and gross pathology. Preliminary conclusions identify the No Observed Adverse Effect Level with daily oral administration of CEQ508 as 10^{11} colony forming units per day. This study will support a 9 month Phase 2b trial, and is providing us with valuable safety information supporting the long-term use of CEQ508.

START-FAP(Safety and Tolerability of An RNAi Therapeutic in FAP) Clinical Trial

Figure 11

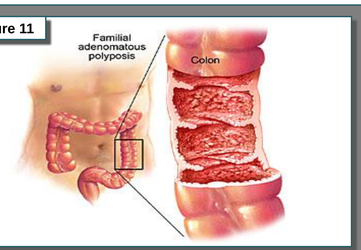


Figure 11:

Familial Adenomatous Polyposis (FAP)

- Rare hereditary disease – 30,000 Pts US; 46,000 EU
- FAP results in the formation of multiple colon polyps
- Polyps appear by age 16 and rapidly progress.
- Colectomy is only available treatment to prevent colon cancer

CEQ508 Development Summary

- Proof of concept in APCmin mouse model
- Safety in two species
- Long term safety established in NHPs
- GMP manufacturing and product release complete
- Lyophilization program initiated
- Orphan Drug Designation granted
- First-in-Man (FIM) enrollment 10 March 2011
- Completion of the Dose Escalation Phase anticipated in Q4 2011 (**Figure 12a**)
- Completion of the Stable Dose Phase planned anticipated in Q1 2012 (**Figure 12b**)

Figure 12a

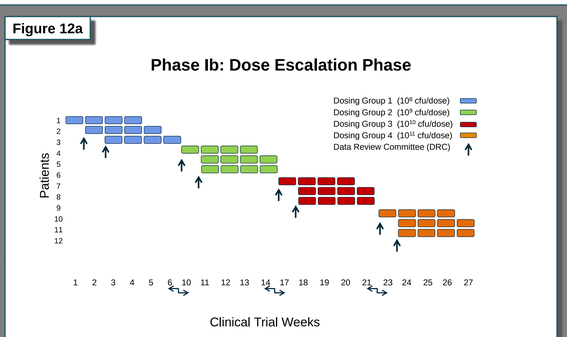
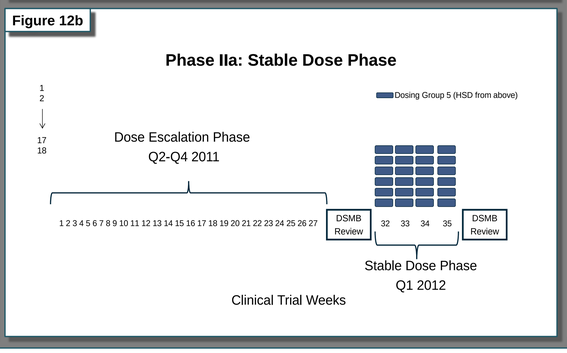


Figure 12b



Summary:

Marina Biotech is developing novel products to deliver RNAi-based treatments to prevent and treat human disease. Our first drug candidate, CEQ508, targeting β-catenin in the intestinal epithelium and has been shown to markedly reduce β-catenin mRNA message levels *in vitro*. Similar results were obtained *in vivo* using the APC^{min} mouse model of FAP. Furthermore, studies in non-human primates confirm that oral *tk*RNAi targeting β-catenin is safe and well tolerated for long term use and is capable of suppressing β-catenin expression in the intestinal tract. Marina Biotech's *tk*RNAi platform represents a novel therapeutic avenue for patients suffering from FAP. The first-in-man START-FAP clinical trial is currently underway with the recently announced first patient enrollment in the Dose Escalation Phase of this two part trial. The primary endpoint is safety and tolerability of daily, oral administration of CEQ508 for 28 days; secondary endpoint includes analysis of β-catenin gene expression levels determined from tissue analysis taken during baseline and endpoint upper and lower endoscopies.