Non-viral DNA-delivery system for familial adenomatous polyposis therapy in a rat model of human colon cancer

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Background

Familial adenomatous polyposis

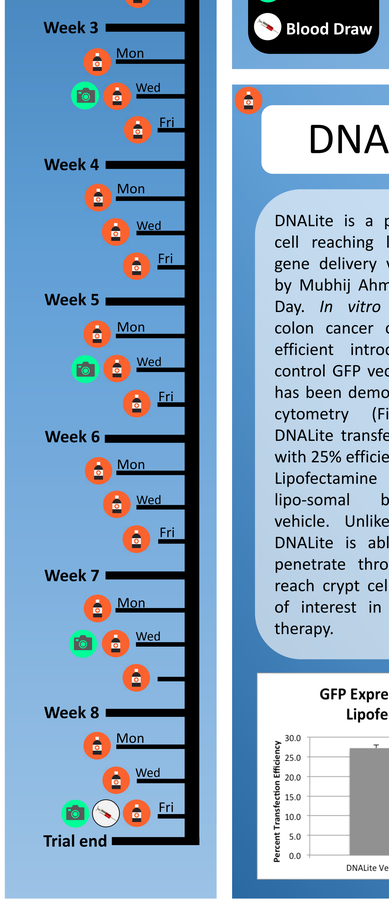
Familial adenomatous polyposis (FAP) is a heterozygous dominant inherited disease causing early onset colorectal cancer (CRC) in 100% of patients. A mutated version of the adenomatous polyposis coli (APC) gene is present in FAP patients, and within 60-80% of CRC cases. In FAP, benign polyps begin formation during the late teen years and frequently progress to carcinomas in patients by their early thirties, resulting in resection of the colonic and duodenal segments.

Hypothesis

Recent studies in model systems have shown that re-established APC expression can induce CRC regression. We hypothesized that a functional APC gene replacement will lead to existing polyp regression and prevent adenomagenesis.

The F344-*Apc*$^{am1137/+}$ PIRC rat model

A mutation in the APC Gene characterizes the PIRC rat model. Phenotypically, the model spontaneously develops polyps throughout the colorectal, and in later stages, small intestinal regions. The polyps follow a similar progression to those affected with FAP (Figure 1).



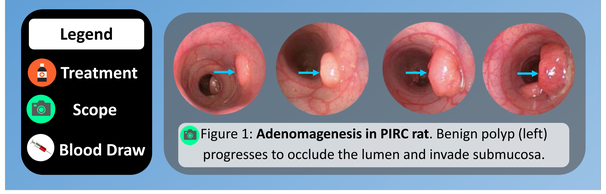


Figure 1: **Adenomagenesis in PIRC rat**. Benign polyp (left) progresses to occlude the lumen and invade submucosa.

DNALite Delivery Vehicle

DNALite is a proprietary stem cell reaching liposomal based gene delivery vehicle designed by Mubhij Ahmad and Timothy Day. *In vitro* transfection of colon cancer cells lines show efficient introduction of the control GFP vector (Figure 2). It has been demonstrated by flow cytometry (Figure 3) that DNALite transfects cells *in vitro* with 25% efficiency compared to Lipofectamine (Lipo), another lipo-somal based delivery vehicle. Unlike Lipofectamine, DNALite is able to effectively penetrate through mucous to reach crypt cells. This makes it of interest in colorectal gene therapy.

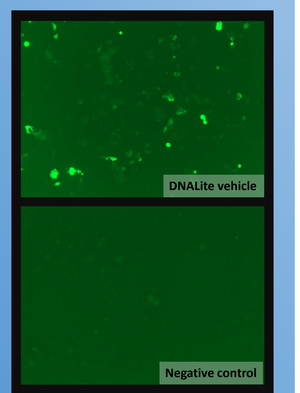


Figure 2: DNALite vehicle was coupled with CMV-GFP. Caco-2 cells were transfected and imaged 48 hrs post-transfection. GFP expression demonstrates gene delivery.

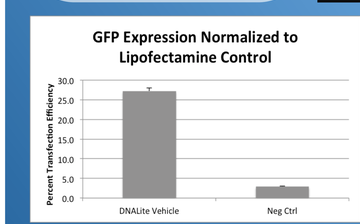
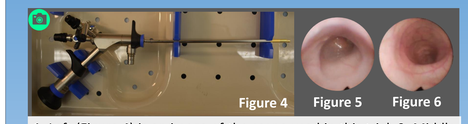


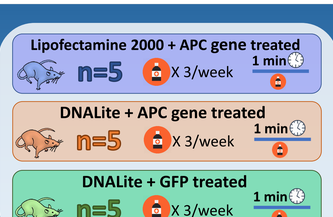
Figure 3: DNALite vehicle and negative control were normalized to the standard transfection agent lipofectamine. Transfection efficiency of the DNALite vehicle was within the same order of magnitude of lipofectamine while retaining mucous penetrating properties.

Trial Design

Rats ranging from 120-210 days were divided into 3 groups (n=5), *viz.* Lipofectamine+APC (positive control), DNALite+APC (test group) and DNALite+GFP (to monitor the efficiency of the DNALite system). The rats are anesthetized with isoflurane and a 0.5-1.0 mL PBS enema is given. After which, 750 µl of the select vehicle is delivered to the distal colon via rectal gavage. Tumor progression is monitored longitudinally throughout the trial by colonoscopies scheduled fortnightly. Blood draws are taken in parallel to monitor changes due to treatment.

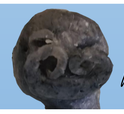


1. Left (Figure 4) is an image of the scope used in this trial. 2. Middle (Figure 5) is a deflated lumen of a PIRC rat 3. Right (Figure 6) is an inflated lumen of a PIRC rat displaying a polyp



Rat Colonoscopies

Colonoscopies are used routinely to assess CRC in rat models. The colonoscope used in this trial is a straight rod pediatric model. The distal colon is inflated with air and maintained by holding off the rectum while the scope is manipulated. Pictures seen are taken in parallel to videos of the procedure.



Development of an *in vivo* measurement system

Eliminating subjectivity in longitudinal polyp monitoring is a challenge as objective methods in polyp sizing are confounded by the nature of colonoscopies. Using a known object to set a scale and further extrapolate the size of its surroundings has shown some consistency, but is labour intensive and often requires additional software. Here we set out to create a pixel to mm conversion to find cross sectional area of the tumour based on the images and a known distance.

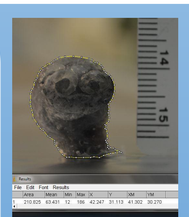


Figure 7: Waxton polyp outlined and measured with Image J

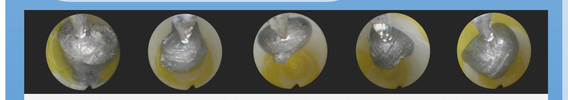


Figure 8: Above are images of the wax polyps taken in the model colon with the colonoscope. Visible is the tip of the biopsy probe, used to set a consistent distance.

Real world cross-sectional areas of irregularly shaped wax polyps were calculated by setting a known conversion to Image J and creating an outline (Figure 7). The wax polyps were positioned in the model colon in the same orientation (as denoted by the dual relief lines). The colonoscope and a biopsy probe was used to capture images and set distance (Figure 8). The *in vitro* scope pictures were processed similarly to the real world images. The cross-sectional area in pixels^2 was used in conjunction with the real world area to determine a conversion factor.

Preliminary longitudinal analysis of polyp transformation

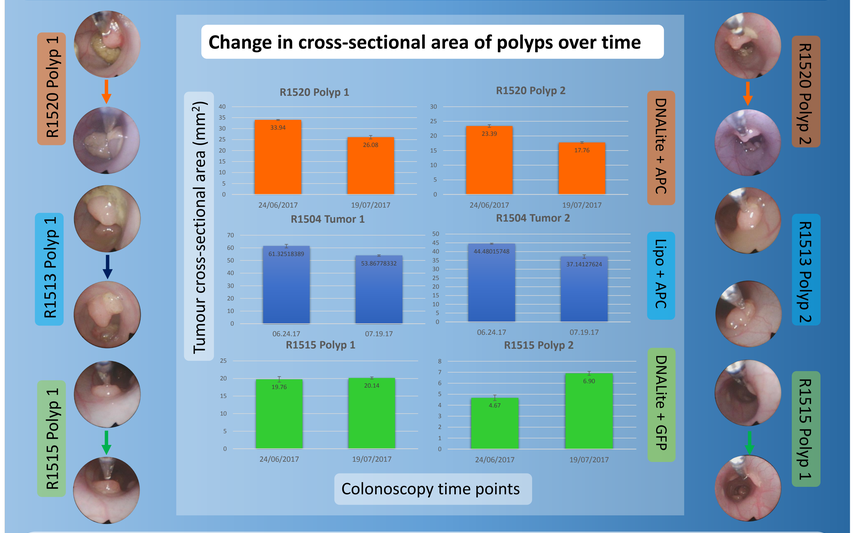


Figure 9: Above are two polyps measured over a three-week period. The cross-sectional area of each polyp was measured and compared to the second-time point. Beside each graph are the images used to process the cross-sectional area. On average polyps are regressing in both the Lipo+APC (2/3 tumors) and the Lite+APC groups (6/7 tumors), while they are maintaining their size or increasing in the Lite+GFP group (7/7 tumors).

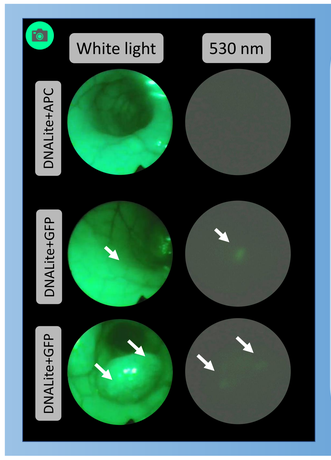
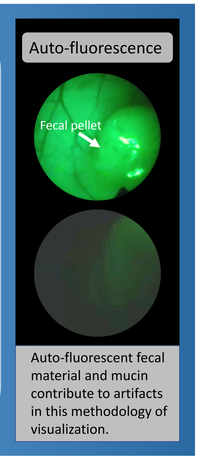


Figure 10: DNALite+GFP and DNALite +APC animals underwent 530 nm filtered light colonoscopies. GFP transfection and expression was assessed *in vivo* by detecting fluorescence. The presence of a fluorescent spot on the images to the left may represent a transfected intestinal crypt or collection of enterocytes. WE observed more fluorescence in tumors that normal epithelium. When compared to the negative GFP control (DNALite + APC group), appreciable fluorescence was visualized in the test group. This indicates *in vivo* transfection and gene expression achieved through the use of DNALite as a delivery vehicle.



Auto-fluorescent fecal material and mucin contribute to artifacts in this methodology of visualization.

Conclusions and future directions

- Visible expression of green fluorescence in the DNALite+GFP group suggest transfection by the DNALite delivery system reaching the crypt base and tumor cells.
- Tumor number and cross-sectional area will be monitored longitudinally via colonoscopy and our developed measurement methodology. We are particularly interested in how tumor changes compare between the DNALite+APC and Lipofectamine+APC groups.
- Initial analysis of polyp cross-sectional area indicate successful APC gene replacement by the DNALite delivery vehicle. The regression in polyp size is promising, especially when compared to the negative control group (DNALite+GFP) that maintained polyp size.
- At 240 days, post-mortem tumor size will be assessed grossly and histologically, and GFP uptake will be visualized through immunohistochemistry. Wet weights of the polyps will also be compared across cohorts.
- Upon completion of the trial, we expect a greater regression of adenoma size in the DNALite+APC group compared to the others.
- If successful, the DNALite delivery system may provide gene therapy options for FAP patients to improve and extend quality of life in this often-debilitating disease.

Acknowledgements

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