

**Progress Report:
Research projects funded by the Childhood Eye Cancer Trust**



All information provided in this report will be kept confidential to the Childhood Eye Cancer Trust, unless stated

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<p>Date:</p>	<p>25-July-2016</p>
<p>Project title:</p>	<p>Use of Aptamers to increase the efficacy of <i>HMGA2</i> targeted therapy in Retinoblastoma</p>
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Childhood Eye Cancer Trust

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Aims / Objectives of project:

Abstract:

Retinoblastoma (RB) is a common childhood eye cancer, where chemotherapy plays an important role in the treatment. Targeted therapy is gaining importance in the treatment of RB. Recent clinical and literature evidences suggest that over expression of non-histone chromatin-binding protein: high mobility group AT hook protein (HMGA) increases cell proliferation in turn contributing to tumor growth. In our previous CHECT funded projects, we have demonstrated that *HMGA2* is up regulated in RB [1]. Subsequently, we utilized RNA interference strategies (RNAi) to knock down *HMGA2* mRNA and studied both gene expression and miRNAs. *HMGA2* RNAi in cultured RB cells reduced cell proliferation and transition in the G1/S phase [2]. In addition, we observed that *HMGA2* regulates many oncogenic and tumour suppressive miRNAs relevant to RB. [3] These results confirmed that *HMGA2* is a potential target for RB therapy. It is worth to note that these biomolecules should reach nucleus to modulate HMGA2 levels. In the present proposal, we aimed to develop strategies for delivering HMGA2 siRNA and also a HMGA Aptamer to target HMGA2 protein in nucleus of RB cells.

Specifically, we had proposed two delivery strategies as presented below:

- (1) The first strategy involves delivery of *HMGA2* siRNA through Aptamer-siRNA chimera. Aptamers are oligonucleotide that bind to a specific target molecule. Aptamers are selected through **SELEX** (systematic evolution of ligands by exponential enrichment) technology. We proposed to use aptamers to one of the surface proteins expressed in RB tumor cells such as EpCAM (epithelial cell adhesion molecule), CD133 or Nucleolin (NCL). We had earlier used EpCAM Aptamer [4,5] and NCL aptamer for delivery to RB cells [6]. The strategy was that the aptamer siRNA chimera after internalization, *HMGA2* siRNA will target the expression of *HMGA2* gene. Specifically, siRNA after internalization will be processed by dicer and result in the depletion of *HMGA2* and eventual cell death. It is important to emphasize that cells that poorly express surface proteins such as EpCAM or CD133 or NCL (normal cells) will be spared and unaffected. Thus, selective killing of RB cells could be achieved and there would be no Off target effects
- (2) In the second delivery strategy, we had to show that the HMGA aptamer has functional property in RB cells. Second was to deliver it to nucleus of the cancer cells using transfecting agents in cell culture. Our hypothesis was to come out with a strategy to deliver HMGA2 a nuclear protein targeting aptamer without transfecting agents so that later it could be taken for *in vivo* work and clinical application. Not much literature existed in this strategy. We proposed to develop a novel antibody mediated delivery of *HMGA2* Aptamer into RB cells. Specifically, we would like to utilize antibody which binds to surface proteins in cancer cells such as EpCAM, CD133 or Nucleolin. i.e (Anti- EpCAM / anti-CD133 monoclonal or anti nucleolin antibody) conjugated with *HMGA2* Aptamer as a delivery candidate. Antibody acts as a target

delivery vehicle and *HMG A2* Aptamer would be released within cytosol of RB cells thereby serving as therapeutic component.

References

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With this aim the objectives of the research proposal are as follows

Objectives for Vision Research Foundation, India	Objectives for University of Missouri-Columbia, USA
1. To fabricate a cell surface binding protein targeting Aptamer HMGA2 siRNA chimera for effective gene delivery to RB cells	3. To fabricate a novel cell specific surface binding antibody–Aptamer conjugate (PEG-Ab- <i>HMGA2</i>) for effective <i>HMGA2</i> Aptamer delivery
2. To investigate the affinity, stability, and intracellular accumulation and efficacy of chimeric Aptamer- <i>HMGA2</i> siRNA in RB cancer cells under different physiological conditions and using cell based assays	4. To investigate the stability, cellular affinity and intracellular accumulation and efficacy of PEG-Ab- <i>HMGA2</i> conjugate in RB cancer cells using cell based assays

Lay summary of project (200- 500 words) (to be completed at the start of project - NOT CONFIDENTIAL - to be displayed on CHECT website):

With advancements in molecular biology, targeted therapy is gaining importance in the treatment of Rb. Aptamers have immense potential in targeted therapy because of their ability to bind to the target with high affinity and specificity. Previous findings showed over-expression of the transcription factor HMGA2 in retinoblastoma transformation. Our previous work also showed the contribution of HMGA2 in cell proliferation and G1/S phase transition in Rb. HMGA2 was also found to regulate many oncogenic and tumour suppressive miRNAs in RB. siRNA and aptamer targeting HMGA2 were able to bring down the proliferation in Rb. But these biomolecules targeting HMGA2 should reach the tumour cells to modulate HMGA2 function with minimal uptake and damage to the normal cells.

In the present proposal, we planned to develop strategies for delivering the HMGA2 siRNA and aptamer targeting HMGA2 to the retinoblastoma cells. Tumour cell specific delivery of the aptamer and siRNA sparing the non-cancerous cell would be the first parameter to be evaluated. We would also investigate that the delivery strategies do not compromise the affinity, stability and functional effect of the siRNA and aptamer targeting HMGA2.

The two novel strategies proposed in this project are: delivery of HMGA2 siRNA through aptamer-siRNA chimeras into the retinoblastoma cells; and developing a novel antibody mediated delivery of aptamer into RB cells.

Summary of progress for CHECT research sub-committee:

- (1) HMGA2 protein regulates retinoblastoma (RB) cancer cell proliferation. We used a stable HMGA DNA Aptamer and showed that it reduced proliferation of the tumor cells. There was regulation of the TGF-beta pathway. There was a synergistic effect with etoposide a chemotherapeutic drug in RB Cells, No significant toxicity was observed in non-neoplastic retinal cells.
- (2) We observed Nucleolin protein expressed in the tumor cell surface and in non neoplastic cells it was expressed in the nucleus. So we used a FDA approved Nucleolin (NCL) Aptamer **AS1411** to deliver the HMGA2 siRNA to RB cells. We used a bispecific linker to conjugate Nucleolin Aptamer to HMGA2 siRNA. We observed that the aptamer chimera reduced the proliferation I the RB cells.
- (3) We successfully synthesized and characterized Nucleolin antibody -Aptamer conjugate. The conjugate was stable. The cellular studies conducted in WERI cell line confirm the surface binding affinity of the conjugate to the nucleolin receptors. The cytotoxic assay results indicate that the possibility of aptamer release from the conjugate is facilitated by the antibody thereby causing cell death.

Lay summary for CHECT research committee:

We have identified proteins that are exclusively expressed by RB tumor cells and not expressed in normal eye cells. One among them is a nuclear protein HMGA2. Hence we sought to target HMGA2 protein to treat retinoblastoma. Earlier we silenced HMGA2 by knocking down its expression at mRNA level and showed deregulated pathways by gene expression studies and also identified microRNAs that are influenced by HMGA2. We also used a DNA Aptamer to functionally block HMGA protein and showed that the aptamer reduced the proliferation in the retinoblastoma cells. However delivery of either the siRNA or the aptamer to the retinoblastoma cells was a challenge without the use of transfecting agents, which we use in the cell culture. Hence we rationalized that use of RB tumor specific cell surface protein delivery model would solve the issue. Thus we collaborated with researchers from Missouri university wherein we used a nucleolin aptamer which bind nucleolin protein which is expressed on the surface in RB cells to deliver the HMGA2 siRNA and the US team used a nucleolin antibody to deliver HMGA2 aptamer to the retinoblastoma cells and we observed that the chemistry is working in *in vitro* model. Thus in this research work we have solved the problem of delivery of a nuclear protein targeting siRNA and aptamer

Details of any publications / presentations of results at meetings etc.

- Nalini V. Deepa P.R. Raguraman R. Khetan V. Reddy M.A. Krishnakumar S. Targeting HMGA2 in Retinoblastoma Cells in vitro Using the Aptamer Strategy. *Ocul Oncol Pathol* 2016;2:262-269 (DOI:10.1159/000447300)
- The data acquired in the project is being written in the form of manuscript for a reputed journal

Presentations:

- Abstract submitted to Asia Arvo Indian Eye Research Group Meeting to be held at Hyderabad in July 2016
- An invention disclosure has been submitted to University of Missouri – Columbia (MU) and a provisional will be filed soon.
- **17UMC004:** Targeted HMGA2 Delivery for Retinoblastoma

Other comments / potential problems / barriers to progress etc.

Indian team: As we faced delivery issues with HMGA2 siRNA in our earlier work (1st year), we adopted a different strategy for delivering HMGA2 siRNA using nucleolin aptamer and bispecific linker sulfo-SMPB. This strategy worked out resulting in effective delivery of HMGA2 siRNA leading to downregulation of HMGA2 specifically in RB cell lines. As these aptamers and siRNA being custom modified for conjugation, it took months for delivery of the materials. This delayed in the progress of our work. In future work we are planning to involve QUARK pharmaceuticals (<http://quarkpharma.com/>) for the novel HMGA2 siRNA modifications. We have already discussed with Dr. Daniel Zurr founder of the company to help in this. Fortunately Quark pharmaceuticals are collaborating with Biocon, India (<http://www.biocon.com/>) for siRNA synthesis for pre clinical trials in India.

US Team: From the results obtained through our current work, we could clearly demonstrate the receptor binding affinity of the NCL Antibody-Aptamer conjugate and therefore we envisage a receptor mediated transport of the aptamer causing cell death. Our future work will focus on determining the mechanistic pathway for the delivery of HMGA2 aptamer through protein regulation studies. We also have an alternate strategy of utilizing F3 peptide (a nucleolin receptor targeting peptide) for conjugating the aptamer and compare the HMGA2 delivery through antibody mediated release. We anticipate that by conjugating with F3 peptide and changing the strategies HMGA2 aptamer can be delivered effectively. Another possibility is to use a pH linker or a nanoparticle based delivery that would help releasing the aptamer inside the nucleus. Upon successful conjugation HMGA2 activity will be monitored by PCR and other indirect methods such as internalization assay, cell proliferation and apoptosis assays.

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