determine fractions of dividing and non-dividing cells, cell cycle analysis was performed on FUdR-treated as well as untreated cells by measuring DNA content with flow cytometry.

Results: Cytotoxicity of combined treatments was higher than the calculated additive effects of either G207 or FUdR. Even at low MOIs (0.01), addition of 10 nM FUdR resulted in complete HCT8 cell kill at day 6 post-infection. Cells treated with 10 nM FUdR showed 3.5-5.5 fold higher overall production of G207 and had 2-10 fold higher β-galactosidase activity at day 3 compared to G207 treatment alone (MOI 0.01). These effects were more pronounced for the chemosensitive cell line HCT8. Cell cycle analysis of FUdR-treated cells demonstrated an increase in S-phase fractions with a corresponding decrease of G0/G1-fractions. S-phase fractions increased from 28% to 73% 24 hours after low (10 nM) or high FUdR exposure (100 nM) in HCT8 cells, while chemoresistant cells showed an S-phase shift ranging from 28-84% with only high dose FUdR.

Conclusion: The present study demonstrates enhanced viral infection and replication of G207 during FUdR exposure resulting in synergy between both agents. Since G207 requires dividing cells for its replication, the ability of FUdR to induce DNA damage and increase the S-phase fraction may be responsible for the observed synergy. This knowledge may be helpful in planning therapy by combining these agents to enhance the effect of regional viral therapy and current chemotherapy in colorectal liver metastases.

794. WITHDRAWN

795. Aggregation of AAV Vectors, its Impact on Liver-directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency

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AAV vectors lead to efficient gene transfer in the liver. AAV vectors are usually delivered to the liver through portal vein or via peripheral vein. To achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes. However, we have recently found that, at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions. The size of aggregates appears to be concentration dependent. In both in vitro and in vivo studies using human A1AT as a reporter gene, it was found that, when the same vector prep was concentrated to different concentrations, the higher the concentration, the larger the aggregates and less efficient the gene transfer. Particularly, when the vector titer reached 5-10 x 10^{13} GCs/ml, gene transfer efficiency was 10-100 folds lower at the same dose as compared to the vector whose titer was 1-5 x 10^{12} GCs/ml. To track vector uptake and pharmacokinetics AAVAlbA1AT was labeled with Cy3, concentrated to different levels and delivered to mouse liver through both portal and tail veins. The trafficking of AAV was monitored microscopically and gene transfer was measured by serum levels of human A1AT. Our preliminary data suggest that large AAV aggregates are sequestered in macrophages and quickly cleared from the liver. A series of formulation studies were performed to prevent and dissolve AAV aggregation. Our preliminary finding indicated that some of our formulations could lead to a 30-50% reduction in the size of aggregates at high vector concentrations. Furthermore, PEGylation of AAV efficiently dissolves AAV aggregates. Comparative studies of the performance of PEGylated and native AAV in vivo is in process. (The first two authors contributed equally to this work.)

796. REP/CAP Gene Amplification Is Crucial for High-yield of rAAV Production in the REP/CAP Cell Line, B50, and is Strongly Inhibited by rAAV-ITRs in a Dose-dependent Fashion

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We recently reported the production of recombinant AAV vectors that are free of replication competent AAV (rcAAV) by using a Rep/Cap cell line, B50, and an Ad-AAV hybrid shuttle vector. It was found that temporal relationship of infection with adenovirus helper and AdAAV hybrid was critical to the AAV productivity. A 24-hour pre-infection with Ad helper was essential. More recently, in an attempt to eliminate the Ad helper virus, replication competent AdAAV hybrids (rcAdAAV) were created in which AAV vector genomes were cloned into E3 region of a replication competent Ad genome. Efforts to optimize rcAdAAV for AAV vector production failed. A systematic study was carried out to understand gene regulation of rep/cap gene expression in B50 cells and develop novel strategies for high yield AAV vector production. Our studies reveal the following. 1) The rep/cap minigene in B50 cells is amplified 10-20 times between 32-48 hrs post adenovirus infection. 2) Such an amplification process can be strongly inhibited by AAV-ITRs delivered by either AdAAV hybrids and AAV vector infection, or plasmid ITR fragment transfection in a dose dependent manner. 3) The inhibitory effect of ITRs leads to significant reduction of Rep proteins, particularly, large Reps and poor yield of AAV vector. 4) In the experiments using plasmid construct pP5Luciferase as a reporter gene, it was found that ITRs could also directly or indirectly inhibit P5 promoter activity in both B50 and 293 cells. Based on our findings, we developed a co- infection method for high yield rAAV productions in B50 cells, which is much simpler and easier to be scaled up. At this point, we speculate that the rep/cap gene amplification is Rep-mediated and the inhibitory effect of ITRs is through at least two levels of regulations: either direct binding /sequestering of Reps and/or other cellular factors such as YY1 and/or direct interaction with the P5 promoter. Experiments are under the way to further understand the cellular mechanism of our observation and to shed light on developing novel strategies for high throughput AAV vector production.

797. AdAAV support High-titer Production of rAAV but not Stable

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It has been difficult to produce a chimeric vector containing both Ad and AAV rep and cap, and to grow such chimeric vectors in 293 cells. By recombination in bacterial host, we were able to make recombinant plasmid AdAAV, and then recombinant AdAAV was successfully generated in 293 cells. High titer of recombinant adeno-associated virus (rAAV) stocks were produced by co-infection of cells with two vectors. One vector was with a chimeric AdAAV, which contained the AAV rep and cap genes and all necessary adenovirus genes required for AAV replication. The second vector was an adenovirus in which the green flourescent protein gene flanked by the terminal repeat ends of adeno-associated virus were cloned into the E1 site of adenovirus. Transfection of 293 cells with these two recombinant adenovirus vector resulted in the large scale, high titer production of recombinant adeno-associated virus expressing GFP with little to no contaminating adenovirus. Recombinant AdAAV expressing rep leads to delay adenovirus replication but the titer was not affected eventurally. However, a deletion within the AAV rep gene occurs after short term passage. These results indicate that co-transfection of 293 cells with 2 recombinant AdAAV is a feasible method to produce high titer and pure rAAV, but passage is limited by non-homologous recombination within the AAV rep gene.