Syllabus for BIMM194 Fall 2012: Genetics and Modern Medicine

Wed. 1:00 – 2:20 PM York 3010
Instructor: Prof. Laurie G. Smith (lgsmith@ucsd.edu)
Available for discussion after class or by appointment

Schedule

Oct. 3: Intro to class

Purpose and scope of class, expectations (mine, yours), grading. How to access research articles including supplementary material, how to get figures into slides. How to find information for your presentation beyond what is recommended in syllabus (papers cited by authors using UCSD ejournals link, PubMed searching, experimental reagents/products manufacturers' websites, Wikipedia). Discuss topics. Discuss document sharing.

Oct. 7 (midnight): deadline to submit your 5 choices (ranked 1-5 in order of preference) of presentation topic/date via email to lgsmith@ucsd.edu

Oct. 10: Identification of fetal aneuploidy via analysis of maternal blood samples (instructor's example presentation)

Background reading for class: JL Simpson (2012). Is cell-free fetal DNA from maternal blood finally ready for prime time? *Obstetrics & Gynecology* 119:883-885.

Research article: HC Fan, YJ Blumenfeld, U Chitkara, L Hudgins and SR Quake (2008). Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc. Nat. Acad. Sci. USA* 105:16266-16271.

Questions to answer during presentation: How can useful information about fetal genotype be obtained in the presence of an excess of maternal DNA from maternal blood? How does does the sequencing method used in this study differ from conventional "Sanger sequencing"? How does it allow relative copy numbers of different parts of the genome (ploidy) to be measured? How accurate are the results of fetal aneuploidy analysis using this approach? Are results from this test available to the prospective parents sooner than those from amniocentesis or CVS? What is the (rather big) mistake in the Simpson piece recommended as background reading?

Oct. 17: gene replacement therapy for hemophilia

Background reading for class:
Kaiser, J. (2011) Gene therapists celebrate a decade of progress. *Science* 334, 29–30.

Research article: Nathwani, A.C., Tuddenham E.G., Rangarajan S. *et al*. (2011) Adenovirus-associated virus and vector mediate gene transfer in hemophilia B. *New England Journal of Medicine*, 365:2357-2365. (Note: useful background for this topic/paper includes familiarity with viral genomes, viral vectors, and background in immunology)

Additional background for presenters:

- Nathwani AC, Gray JT, Ng CY, et al. (2006). Self complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. *Blood* 107:2653-2661.
- Nathwani AC, Gray JT, McIntosh J, et al. (2007) Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. *Blood* 109:1414-1421.
- D.J. Roberts (2012) New genes for old: successful gene therapy for haemophilia B. *Transfusion Medicine* 22:3-4.

Questions to address in presentation: What are some of the reasons why gene therapy has not been more successful up to now? What is hemophilia, how it is typically treated currently? How does the "adenovirus associated virus" used in this study work as a vector (see 2006 Blood paper)? Where does viral vector go after injection and how do its effects persist for as much as 16 months after injection (see 2007 Blood paper)? Why did the FIX levels fluctuate as much as they did in some study participants? Did the therapy work for all study participants? Was the benefit of gene therapy proportional to dose of vector? Are there hemophilia sufferers for whom this treatment would not work, and if so, why? Were there adverse reactions or other problems that could limit the clinical utility of this therapy? What is the difference between phase I, II and III clinical trials and which kind was this?

Oct. 24: **gene suppression therapy for Huntington's Disease**

Background for class: http://en.wikipedia.org/wiki/Huntington%27s_disease

Research paper: RL Boudreau, JL McBride, I Martins, S. Shen, Y Xing, BJ Carter, and BL Davidson (2009). Non allele-specific silencing of mutant and wild type Huntingtin demonstrates therapeutic efficacy in Huntington's Disease Mice. *Molecular Therapy* 17:1053-1063 (Note: useful background for this paper includes familiarity with microarray analysis and mechanisms of double stranded RNA-mediated gene silencing).

Additional background for presenters: LM Watson and MJA Wood (2012). RNA therapy for polyglutamine neurodegenerative diseases. *Expert Reviews in Molecular Medicine* 14:1-23.

Questions to address in presentation: What is the genetic basis of Huntington's Disease (pattern of inheritance, underlying mutation, how mutation produces symptoms)? What is "Huntingtin"? How is Hungtinton's Disease currently treated/managed (see Wikipedia entry on HD)? How was the mouse model generated that is used to test experimental therapies? What are the relative merits or problems with allele-specific vs. non allele-specific htt silencing? What is the difference between a shRNA and a miRNA and why does this study focus on miRNA? How is the miRNA delivered to affected tissues? Did the miRNA suppress the function of both wild type and mutant versions of HTT as expected? What effects did this have on the Huntington's Disease-like symptoms of the treated mice? What is needed to move the findings of this study into the clinic?

Oct. 31: antisense oligonucleotide therapy for familial hypercholesterolemia

Background reading for class: http://www.isispharm.com/Pipeline/Therapeutic-Areas/Cardiovascular.htm#Mipomersen (see intro and section on KYNAMRO™)

Research article: FJ Raal, RD Santos, DJ Blom, et el. (2010). Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet* 375:998-1006.

Additional background for presenters:

- CF Bennett and EE Swayze (2010). RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu. Rev. Pharmacol. Toxicol. 50:259-293.
- KG Parhofer (2012). Mipomersen: evidence-based review of its potential in the treatment of homozygous and severe heterozygous familial hypercholesterolemia. *Core Evidence* 7:29-38.

Questions to address in presentation: What are LDL, HDL and ApoB? What is the significance of lipoproteins in normal physiology and cardiovascular disease? What is the genetic basis of familial hypercholesterolemia? What is mipomersen and who needs it? How does the interaction between mipomersen and apoB mRNA lead to degradation of this RNA and LDL reduction? What is the chemical modification of the mipomersen oligonucleotide and why is it used? (For the latter two questions, see Bennett and Swayze review) How well does mipomersen work as a LDL lowering drug? What problems/side effects does it have that could limit its clinical utility? What clinical trial phase was this study?

Nov. 7: Analysis of EGR receptor alleles in lung cancer guiding chemotherapy

Background for class (and presenters):

- Clodagh O'Shea's cancer lecture for BICD100 (to be distributed)
- MR Stratton (2011). Exploring the genomics of cancer cells: progress and promise. *Science* 331:1553-1558. Important foundation for the next few weeks!

Research paper: W Pao et al. (2004). EGF receptor mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Nat. Acad. Sci. USA* 101:13306-13311.

Additional background for presenters:

- http://www.cancer.gov/cancertopics/druginfo/erlotinibhydrochloride
- http://www.cancer.gov/cancertopics/druginfo/gefitinib
- D Stuart and WR Sellers (2009). Linking somatic genetic alterations in cancer to therapeutics. *Curr. Op. Cell Biol.* 21:304-310.

Questions to address in presentation: What is the EGF receptor and how does the wild type version normally work? What are gefitinib and erlotinib and how do they work as chemotherapy agents? Are these already in use as chemotherapy drugs (for what cancers?) What is the genetic difference between lung cancers that respond to these drugs and those that don't? What connection between smoking and sensitivity to these chemotherapy drugs is revealed by this study? What are the clinical implications of this study regarding who should receive gefitinib or erlotinib treatment for lung cancer?

Nov. 14: **discovery of small molecule inhibitor of the B-Raf oncogene in melanoma**

Background for class:

- MR Stratton (2011). Exploring the genomics of cancer cells: progress and promise. *Science* 331:1553-1558. (again)
- http://abcnews.go.com/Health/CancerPreventionAndTreatment/drug-deadly-melanoma/story?id=15767285#.UFSNRhje6eI (see text and first two videos - second video follows first automatically; look at the books on the shelf behind the first person interviewed – see anything familiar?)

Research paper: J Tsai, JT Lee et al. (2008). Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc. Nat. Acad. Sci. USA* 105:3041-3046. (Note: useful background for this paper: structural biology = protein structure analysis)

Additional background for presenters:

- http://www.cancer.gov/cancertopics/druginfo/fda-vemurafenib
- D Stuart and WR Sellers (2009). Linking somatic genetic alterations in cancer to therapeutics. *Curr. Op. Cell Biol.* 21:304-310.

Questions to address in presentation: What genetic lesion (mutation) in melanoma is targeted by vemurafenib, and how does this mutation contribute to oncogenic transformation? What is the evidence that this mutation plays a major role in melanoma formation? How common is it in melanoma? How was vemurafenib identified as a promising V600E B-Raf inhibitor? How selective is vemurafenib (PLX4720) for inhibition of V600E vs. wild type B-Raf in the various assays employed? How effective is the drug at inhibiting the growth of melanoma cells in vivo? How effective was vemurafenib in treatment of metastatic melanoma in clinical trials?

Nov. 21: **Targeted analysis of oncogene alleles guiding cancer treatment plans**

Background for class and presenters: MR Stratton (2011). Exploring the genomics of cancer cells: progress and promise. *Science* 331:1553-1558. (again)

Research paper: D Dias-Santagata et al. (2010). Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol. Med.* 2:146-158. (Note: at least one person on the team for this presentation needs to tackle the job of explaining to the class how the SNaPshot assay works for identifying mutations in cancer cells – best for someone with a strong background in molecular biology methods e.g. PCR and sequencing).

Additional background for presenters:
Lindblad-Toh et al. (2000) Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. *Nat. Genet.* 24:381-386 (original description of the method that was commercialized by Applied Biosystems as the SNaPshot assay).

Questions to address in presentation: What was the goal of this study? Why did the authors choose SNaPshot technology to analyze tumor genotypes? How does it work (how are genetic differences between normal and tumor cells detected and visualized)? What genes were targeted for analysis and why? How was the information obtained through the analyses they did used to improve patient treatment?

Nov. 28: **PCR-based assay of patient-specific chromosomal rearrangements to monitor tumor burden in cancer patients**

Background for class and presenters: MR Stratton (2011). Exploring the genomics of cancer cells: progress and promise. *Science* 331:1553-1558. (last time!)

Research paper: RJ Leary et al. (2010). Development of personalized tumor biomarkers using massively parallel sequencing. *Science Translational Medicine* 2(20):20ra14 (pg. 1-7). (Note: as for the topic above, at least one person in the group will need to tackle explaining the technical aspects of this study to the class: the PARE approach and digital PCR – best for someone with a strong background in molecular biology methods).

Additional background for presenters:

- KJ McKernan et al. (2009). Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding. *Genome Res.* 19:1527-1541. This is ref. 15 in Leary et al. and sounds like the first description of the paired end read approach for identifying rearrangements.
- http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/PCR/real-time-pcr/real-time-pcr-applications/digital-pcr.html# (info about digital PCR - watch video, read text, click on link "see how digital PCR and real time PCR compare" and read the info there too)

Questions to address in presentation: What kind of genetic alteration is being identified via PARE? What is the purpose of the PARE analysis? How were chromosomal rearrangements identified in patient tumors (this will involve explanation of "mate paired tags")? How were rearrangements confirmed? Once confirmed, how were rearrangements turned into an assay for the presence of tumor DNA in patient blood? What is "digital PCR" used for quantification of tumor DNA in patient blood samples and how is this different from more standard quantitative PCR assays such as real time qPCR (see URL above)? What did the digital PCR analysis reveal about the response of the chosen patient to treatment of his/her tumor? What are the advantages and disadvantages of the approach described in this study to monitoring response to treatment compared to conventional methods?

Dec. 5: Whole exome sequencing as a diagnostic tool

Background for class and presenters:

- R Drmanac (2012). The ultimate genetic test. *Science* 336:1110-1112.
- LR Brunham and MR Hayden (2012). Whole genome sequencing: the new standard of care? Science 336:1112-1113.

These short back to pack perspective pieces in *Science* represent contrasting views of the value of whole genome (or exome) sequencing in clinical medicine.

Research article: EA Worthey et al. (2011). Making a definitive diagnosis: sequencing of exomes in child with inflammatory bowel disease. *Genetics in Medicine* 13:255-262.

Additional background for presenters:
http://www.nimblegen.com/products/lit/05227887001_SeqCapBroch_Oct2011.pdf - brochure with good explanation and illustrations, but doesn't discuss the sequence capture array used by Worthey et al. This is explained pretty well in the first 4.5 minutes of the Jan. 15, 2009 webinar

by Dan Burgess found at http://www.nimblegen.com/news/events/webinar/index.html. Note that the "probes" they speak of on the microarray are oligonucleotides representing human exon DNA sequences.
Also see TJ Albert, Direct selection of human genomic loci by microarray hybridization. *Nature Methods* (2007) 4:903-905.

Questions to address in presentation: Why was this child singled out for close scrutiny of his genome? Was there a priori evidence that his disorder was heritable? What is the "exome"? How are exomes selectively sequenced (as opposed to sequencing all genomic DNA – answering this will involve explanation of how the Nimblegen sequence capture/exome array works)? How did the authors get from initial identification of 16,124 changes relative to the reference human genome sequence to identification of the putative causal mutation of the child's disease? What is XIAP and what is its function (see references cited as #13-15)? How was the XIAP mutation confirmed as a likely culprit in this child's disease? What treatment decision was made based on the exome analysis and why? What was the outcome of this treatment?

Expectations and grading:

Grades will be determined as follows (see further explanation of each component, below):
In class presentation: 40 points
Attendance and participation: 30 points
Write ups of other students' presentations: 30 points

Student presentations: Students will work in teams to present the research article of the week along with background information (each student will be on one team of 3-4 students). Work should be divided roughly equally among members of the team, for example (for a team of 3): one person presenting background, one presenting methods and results, and one discussing the conclusions and implications. Each person will be evaluated separately on the depth, accuracy and clarity of their presentation. I will meet with each group of students once before their presentation; a representative from each group should schedule this meeting with me after determining when all members of the group are available. This meeting should be no later than the Monday before the presentation and better yet the week before. These meetings are not mandatory, however, if the group feels it is unnecessary.

Students should send Prof. Smith (lgsmith@ucsd.edu) a ranked list of their top 5 choices for topics/presentation dates by midnight Sun. Oct. 7. She will work out the teams/assignments and communicate those to students no later than class time Wed. Oct. 10.

General guidelines for presentations: Background info should include general information about disease/health condition addressed by the research, cause and prevalence, other therapeutic options (need for new/better treatment options), molecular biology/cell biology/genetics background info needed to understand therapeutic or diagnostic approach utilized in the research paper. Typically this background will need to go beyond what is presented in the introduction of the research paper since this introduction will assume that most readers already have this background and intro lengths are limited. There should always be some discussion of methods used in the research, but this should be coupled with discussion of the corresponding experimental results rather than be a separate section of the presentation as it is in the research article. Each figure of the research article should be displayed, explained and discussed.

Presentation of supplementary figures is optional – you should decide based on importance to the story whether to include them. The presentation should wrap up with a discussion of the results, conclusions and implications of the study. Be sure to address what are the impacts of the findings on the diagnosis and treatment of human disease! For each topic, a list of up to ten specific questions is provided in the syllabus for presenters to address – be sure to address them somehow, somewhere in the presentation.

Attendance and participation: Attendance and active participation in class discussions are expected of all students. Participation means asking questions and contributing comments and thoughts to discussions taking place on days you are not presenting. Quantity matters but not as much as quality (you should speak up several times throughout the quarter but will not receive more and more credit for speaking up during every class period – be aware that others need to have the opportunity to contribute to the discussion as well!). Everyone gets one "free" absence (you can miss class once without this counting against your attendance/participation score).

Write-Ups of other students' presentations: A list of specific questions for each group of students to answer in their presentation is provided in this syllabus. You will need to hand in a 1-2 page (single spaced) summary with brief answers to all but one (you can skip one and still get full credit) of these questions for five presentations this quarter. Thus, you should examine the list of questions in advance of each presentation and make sure you learn the answers in class. If you feel you did not get a satisfactory answer, feel free to ask the presenters! However, no student in the class will be obliged to answer these questions outside of classtime (e.g. via email), so please focus on getting the answers in class. Summaries are due by 5pm on Sunday after the presentation (email to lgsmith@ucsd.edu). If two students hand in summaries with strikingly overlapping/duplicated wording, neither will be accepted. Thus, if you collaborate with other students on your write up be sure to put the final summary in your own words. To receive credit for your write ups, you must hand in all five (each is worth 6 points, but only if you hand in all five).