Key for Bi188 HW2

1. [Keep in mind that Q1 is supposed to be a model trial-and-error question, so it's "open" as long as your model works. This is but one possibility.]

A) A is autosomal dominant. Mrs. Smith does not carry A.

B) No, because one of Mr. Smith's sister carries A but not the disease phenotype.

C) At least one copy of A and one copy of B (both autosomal) are needed to express phenotype of the disease. Mr. Smith's untested sister is probably a/+ and +/+. Mrs. Smith is probably +/+ and either b/+ or b/b.

2A-0.5) Multiple acceptable answers. For example: The tumor with 2x somatic mutation has acquired a secondary mutation in a DNA ligase, causing a higher error rate because mutations accumulate more quickly during cell division.

2B-1.0) Multiple acceptable answers - 0.5 points for each of two. For example: a translocation could bring an enhancer to a proto-oncogene, causing it to have increased expression (and become an oncogene). A translocation could also remove a repressor from near a proto-oncogene or fuse it with the promoter of another gene (like BRC/ABL) Or: a gene duplication event could cause the proto-oncogene(s) to have higher expression in the cell. Chromothrypsis can cause many of these events to occur too (by causing an oncogene to have many copies on smaller chromosomes or by causing an enhancer to copy itself and activate several new genes).

2C) (0.5) What additional criteria can be imposed to focus on inherited alleles? Multiple answers: Large-scale: focus on families where many have the same cancer, focus on young patients with the cancer rather than old, small-scale: do SNP analysis or RNA-Seq for patient and family to determine if there are any genetic changes that could both be inherited and causative of the cancer

(0.5) How can you identify the breast cancer tumor suppressor genes in comtemporary times but without doing whole gneome sequencing? Gel electrophoresis after enzymatic cut to look for gene duplications, giemsa stain and karyotyping to look for chromosomal abnormalities, etc.

3. [Sample answers, length can vary]

3.(1) you should get samples of the child's tumor cells (infected white blood cells), samples of the child's healthy white blood cells if available (bone marrow maybe) and if not, sibling or parent healthy white blod cells. You would also need to analyze the parent white blood cells to make a determination about the genetics of the patient's lymphoma.

After the samples have been collected, whole-genome sequencing will show most of the SNV's that the child's tumor cells contain. However, only one or a few of those mutations will have anything to do with causing the cancer because so many cancers accumulate secondary mutations. First, compare the child's tumor cells to the child's healthy cells to find which mutations are inherited / in all of the patient's cells. Next, rule out the silent mutations by identifying the mutations that are in exons or CRM's relevent to white blood cells (ideally by doing an RNA-Seq + ChIP with relevant factor like pol2 or Eprotein, though they might not think of ChIPs for the midterm).

At the end, you will have a handful of candidate mutations that are probably not inherited and that seem biologically relevant to the cancer. As a physician, you must weigh the urgency of the disease against the time it will take to do more testing. Perhaps you could look in the RNA-Seq or some ChIP-Seq, DNAse hypersensitivity data, or methyl-Seq to see if there are any changes from healthy cells in the region of your mutations. Finding such changes might mean the mutation is causative... or they may merely correlate with the mutation. The only way to tell for sure is to do an experiment. For CRM mutations, a transfection assay or something else that would test the ability of the region to bind factors and affect transcription would be best. For exome mutations, the patient's RNA-Seq should tell you which genes are supposedly affected. You should do an experiment, though, to determine if the pertinent proteins or RNA's, not just the data, look strange. For example, if a protein looks like it's missing an exon in RNA-Seq, do a pull-down for that protein in the cancer cells and verify that the protein is missing (ubiquitinated) or the wrong size/shape.

3.(2) For this part, analyze the DNA sequence of the patient's healthy cells and all of the family members' healthy cells. First, find SNV's for each person. Then for each of the patient's SNV's, determine if there is an identical SNV in one of their parents. Doing this will cause you to end up with a selection of good but not final candidate inherited mutations at the end, which you must then verify or test as in part 3(1)above.