This Workflow is a continuation of Workflow Part 2 Part A, which refers to the poster in Workflow Part 2 Part B, and contains a description of results from virtual experiments presented in a poster at the NIH MSM meeting in 2014 (see Workflow Part 3 Part B). This 2014 poster contains panels similar to the 2013 poster; describing liver lobule physiology and zonation (poster panel a), the spatial and temporal pattern of necrosis caused by AILI (poster panel b), and the in silico liver lobule analog we use to conduct our virtual experiments (poster panel c). In the 2013 poster, the referent (i.e. wet-lab) experimental context was perfused liver lobules and the validation target was a tipping point scenario where significantly more liver damage was produced in Zone 3 near the CV than in Zone 2 and Zone 1 near the PV. Our objective is to implement mechanistic hypotheses that achieve the following validation target for the spatial and temporal pattern of AILI: necrosis occurs first in hepatocytes close to CV (Zone 3) and then progresses outward toward PV. Therefore, we implemented additional mechanisms to our liver lobule analog. First, NAPQI produces two types of Damage each of equal probability; one maps to non-mitochondrial damage (D1) and the other maps to mitochondrial damage (D2), which is significantly more potent. Second, mitochondrial damage can amplify further mitochondrial damage through mitochondrial dysfunction causing the accumulation of reactive oxygen species. Third, each Damage type can be repaired by a Repair mechanism but with a different spatial (i.e. PV-to-CV) parameterization. Last, if mitochondrial damage within a hepatocyte breaches a threshold then this triggers the cell's death with a time delay selected randomly within an interval. All these mechanisms are graphically represented in poster panel e with parameterizations in poster panel p. A more detail description of experimental results with these mechanisms has been published (see Publications on this AILI Simtk site). Because of the many in vivo experiments on mouse strains available from the literature and providing targeted attributes (i.e. phenomena to simulate), we wanted to replicate this referent experimental context virtually. Therefore, we structural modified the lobule analog to include a “body” compartment, in which a ip dose of APAP (or necrosis inhibitor INH) can be administered (poster panel d); therefore, forming an in silico mouse analog. After each mechanism implementation and structural modification, the analog must be iteratively refined to not only achieve new validation targets but also to insure that previously validated targets are no compromised. This Iterative Refinement (IR) Protocol (poster panel f) is a combination of the scientific method and software engineering. Briefly, for each targeted attribute parsimoniously implement a mechanistic hypothesis, then test this hypothesis by performing experiments involving changing mechanistic parameters. If the phenotype (i.e. measure data or behavior) of the analog matches within error data measured from the referent system according to a prespecified similarity criterion, then the targeted attribute has been validated; if not, then the analog has been falsified, afterwards the granularity of mechanism must increase or a different mechanism implemented. After many iterations, a mouse analog with parameterizations (poster panel p) similar to the previous lobule analog achieved the validation target N, D1, D2 in Zone 3 >> Zone 2 > Zone 1 (poster panels g, h, & i, respectively) , along with the additional validation targets of more cell deaths near CV in Zone 3 (poster panel k & o), cell death progress outward from CV toward PV over time (poster panel j & o), and characteristic APAP clearance and metabolite formation (poster panel m). Furthermore, another validation target was that 30 minutes after an APAP dose an IP dose of a necrosis inhibitor INH (blocks Jun N-terminal kinase) significantly decreases necrosis. To achieve this objective, we implemented a inhibitor mechanism (bottom of poster panel e) in which if a cell has been triggered to die and inhibitor is present, then the death event has a chance to be canceled with a probability that depends on the amount of inhibitor to the amount of damage and the potency of the inhibitor. With a dose of INH, an approximate 50% decrease in cell death was achieved comparable to decreasing the dose of APAP by 50% (poster panel n).