BAY-GLUT1 inhibited significant accumulation of cholesterylesters as early as 6 hours after treatment in HeLa-MaTu cells, but only after 48 hours in DLD-1 cells. (Figure 5) BAY-GLUT1 treatment led to significant reduction of glycerol-3-phosphate, lysocephatidylcholine (LPC), and lysophosphatidylethanolamine (LPE) at 6 hours post-treatment in HeLa-MaTu cells (Figure 5) and 24 hours in DLD-1 cells (Figure 5). BAY-GLUT1-induced changes in lipid metabolism: cholesterylesters - inhibition of cholesterylester biosynthesis to allow accumulation of toxic oxysterols • Potential survival strategy: β-oxidation

2-Hydroxyglutaric acid: key discriminator for HeLa-MaTu and DLD-1 cells • DLD-1 tends to have a gain-of-function mutation in isocitrate dehydrogenase 1, allowing the conversion of α-KG into 2-Hydroxyglutaric acid (2-HG) (Figure 6) • BAY-GLUT1-induced changes in glucose metabolism: cholesterylesters, β-oxidation, and lipid metabolism. • BAY-GLUT1 treatment led to significant reduction of glycerol-3-phosphate, lyso-cephatidylcholine (LPC), and lysophosphatidylethanolamine (LPE) at 6 hours post-treatment in HeLa-MaTu cells (Figure 5) and 24 hours in DLD-1 cells (Figure 5). BAY-GLUT1-induced changes in lipid metabolism: cholesterylesters - inhibition of cholesterylester biosynthesis to allow accumulation of toxic oxysterols • Potential survival strategy: β-oxidation

SUMMARY & CONCLUSIONS • BAY-GLUT1 induced similar metabolic reactions in both cancer cell lines, with HeLa-MaTu showing more pronounced early responses • Potential explanations for BAY-GLUT1 susceptibility include: 1) lower baseline glycolytic activity of HeLa-MaTu cells; 2) preference of DLD-1 for utilizing glutamine over glucose; and 3) accumulation of 2-HG in DLD-1 leading to metabolic reprogramming • Possible survival mechanisms induced by BAY-GLUT1 may suggest the following: Preclinical investigators: β-oxidation • Inhibitors of de novo biosynthesis of phospholipids Table 3. Summary of metabolic features of BAY-GLUT1-treated cancer cell lines.