

1. Support for Biological Plausibility of KERs	
<p>MIE1 => KE1:</p> <p>Increases in cellular ROS leads to porcupine-induced Wnt secretion and Wnt signaling activation</p>	<p>Biological Plausibility of the MIE1 => KE1 is moderate.</p> <p>Rationale: Increases in cellular ROS caused by/causes DNA damage, which will alter several signaling pathways including Wnt signaling. ROS stimulate inflammatory factor production and Wnt/β-catenin signaling (Vallée & Lecarpentier, 2018).</p>
<p>MIE2 => KE1:</p> <p>Chronic ROS leads to porcupine-induced Wnt secretion and Wnt signaling activation</p>	<p>Biological Plausibility of the MIE2 => KE1 is moderate.</p> <p>Rationale: Sustained ROS increase caused by/causes DNA damage, which will alter several signaling pathways including Wnt signaling. Macrophages accumulate into injured tissue to recover the tissue damage, which may be followed by porcupine-induced Wnt secretion. ROS stimulate inflammatory factor production and Wnt/β-catenin signaling (Vallée & Lecarpentier, 2018).</p>
<p>KE1 => KE2:</p> <p>Porcupine-induced Wnt secretion and Wnt signaling activation leads to beta-catenin activation</p>	<p>Biological Plausibility of the KE1 => KE2 is moderate.</p> <p>Rationale: Secreted Wnt ligand stimulates Wnt/β-catenin signaling, in which β-catenin is activated. Wnt ligand binds to Frizzled receptor, which leads to GSK3β inactivation. GSK3β inactivation leads to β-catenin dephosphorylation, which avoids the ubiquitination of the β-catenin and stabilize the β-catenin (Clevers & Nusse, 2012).</p>
<p>KE2 => KE3:</p> <p>beta-catenin activation leads to Epithelial-mesenchymal transition (EMT)</p>	<p>Biological Plausibility of the KE2 => KE3 is moderate.</p> <p>Rationale: β-catenin activation, of which mechanism include the stabilization of the dephosphorylated β-catenin and translocation of β-catenin into the nucleus, induce the formation of β-catenin-TCF complex and transcription of transcription factors such as Snail, Zeb and Twist (Clevers & Nusse, 2012) (Ahmad et al., 2012; Pearlman et al., 2017; Sohn et al., 2019; Yang W et al., 2019).</p> <p>EMT-related transcription factors including Snail, ZEB and</p>

	<p>Twist are up-regulated in cancer cells (Diaz et al., 2014). The transcription factors such as Snail, ZEB and Twist bind to E-cadherin (CDH1) promoter and inhibit the CDH1 transcription via the consensus E-boxes (5' -CACCTG-3' or 5' -CAGGTG-3'), which leads to EMT (Diaz et al., 2014).</p>
<p>KE3 => AO: Epithelial-mesenchymal transition (EMT) leads to treatment-resistant gastric cancer</p>	<p>Biological Plausibility of the KE3 => AO is moderate. Rationale: Some population of the cells exhibiting EMT demonstrates the feature of cancer stem cells (CSCs), which are related to cancer malignancy (Shibue & Weinberg, 2017; Tanabe, 2015a, 2015b; Tanabe et al., 2015). EMT phenomenon is related to cancer metastasis and cancer therapy resistance (Smith & Bhowmick, 2016; Tanabe, 2013). Increase expression of enzymes that degrade the extracellular matrix components and the decrease in adhesion to the basement membrane in EMT induce the cell escape from the basement membrane and metastasis (Smith & Bhowmick, 2016). Morphological changes observed during EMT is associated with therapy resistance (Smith & Bhowmick, 2016).</p>
2. Support for essentiality of KEs	
<p>KE1: Porcupine-induced Wnt secretion and Wnt signaling activation</p>	<p>Essentiality of the KE1 is moderate. Rationale for Essentiality of KEs in the AOP: The Wnt signaling activation is essential for the subsequent β-catenin activation and cancer resistance.</p>
<p>KE2: beta-catenin activation</p>	<p>Essentiality of the KE2 is moderate. Rationale for Essentiality of KEs in the AOP: β-catenin activation is essential for the Wnt-induced cancer resistance.</p>
<p>KE3: Epithelial-mesenchymal transition (EMT)</p>	<p>Essentiality of the KE3 is moderate. Rationale for Essentiality of KEs in the AOP: EMT is essential for the Wnt-induced cancer promotion and</p>

	acquisition of resistance to anti-cancer drug.
3. Empirical support for KERs	
<p>MIE1 => KE1:</p> <p>Increases in cellular ROS leads to porcupine-induced Wnt secretion and Wnt signaling activation</p>	<p>Empirical Support of the MIE1 => KE1 is moderate.</p> <p>Rationale: Production of ROS by DNA double-strand break causes the tissue damages (Gao et al., 2019).</p> <p>ROS signaling induces Wnt/β-catenin signaling (Pérez et al., 2017).</p>
<p>MIE2 => KE1:</p> <p>Chronic ROS leads to porcupine-induced Wnt secretion and Wnt signaling activation</p>	<p>Empirical Support of the MIE2 => KE1 is moderate.</p> <p>Rationale: Production of ROS by DNA double-strand break causes the tissue damages (Gao et al., 2019).</p> <p>ROS signaling induces Wnt/β-catenin signaling (Pérez et al., 2017).</p>
<p>KE1 => KE2:</p> <p>Porcupine-induced Wnt secretion and Wnt signaling activation leads to beta-catenin activation</p>	<p>Empirical Support of the KE1 => KE2 is moderate.</p> <p>Rationale: Dishevelled (DVL), a positive regulator of Wnt signaling, form the complex with FZD and lead to trigger the Wnt signaling together with Wnt coreceptor low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) (Clevers & Nusse, 2012; Jiang et al., 2015).</p> <p>Wnt binds to FZD and activate the Wnt signaling (Clevers & Nusse, 2012; Janda et al., 2012; Nile et al., 2017). Wnt binding towards FZD induce the formation of the protein complex with LRP5/6 and DVL, leading to the down-stream signaling activation including beta-catenin (Clevers & Nusse, 2012).</p>
<p>KE2 => KE3:</p> <p>beta-catenin activation leads to Epithelial-mesenchy</p>	<p>Empirical Support of the KE2 => KE3 is moderate.</p> <p>Rationale: The inhibition of c-MET, which is overexpressed in diffuse-type gastric cancer, induced increase in phosphorylated β-catenin, decrease in β-catenin and Snail</p>

mal transition (EMT)	<p>(Sohn et al., 2019).</p> <p>The garcinol, that has anti-cancer effect, increases phosphorylated beta-catenin, decreases β-catenin and ZEB1/ZEB2, and inhibit EMT (Ahmad et al., 2012).</p> <p>The inhibition of sortilin by AF38469 (a sortilin inhibitor) or small interference RNA (siRNA) results in decrease in β-catenin and Twist expression in human glioblastoma cells (Yang W. et al., 2019).</p> <p>Histone deacetylase inhibitors effect on EMT-related transcription factors including ZEB, Twist and Snail (Wawruszak et al., 2019).</p> <p>Snail and Zeb induces EMT and suppress E-cadherin (CDH1) (Batlle et al., 2000; Diaz et al., 2014; Peinado et al., 2007).</p>
<p>KE3 => AO:</p> <p>Epithelial-mesenchymal transition (EMT) leads to Treatment-resistant gastric cancer</p>	<p>Empirical Support of the KE3 => AO is moderate.</p> <p>Rationale: EMT activation induces the expression of multiple members of the ATP-binding cassette (ABC) transporter family, which results in doxorubicin resistance (Saxena et al., 2011; Shibue & Weinberg, 2017).</p> <p>TGFβ-1 induced EMT results in the acquisition of cancer stem cell (CSC) like properties (Pirozzi et al., 2011; Shibue & Weinberg, 2017).</p> <p>Snail-induced EMT induces the cancer metastasis and resistance to dendritic cell-mediated immunotherapy (Kudo-Saito et al., 2009).</p> <p>Zinc finger E-box-binding homeobox (ZEB1)-induced EMT results in relief of miR-200-mediated repression of programmed cell death 1 ligand (PD-L1) expression, a major inhibitory ligand for the programmed cell death protein (PD-1) immune-checkpoint protein on CD8⁺ cytotoxic T lymphocyte (CTL), subsequently the CD8⁺ T cell immunosuppression and metastasis (Chen et al., 2014).</p>