Table 1: LTin Assumptions

Agent	State	Model	Assumptions
LTin	Random Movement on Tract Surface	Domain	• There is no attractive influence on an LTin cell – any contact with RET Ligand Expressing Cells will occur randomly
		Platform	• Each cell is assigned a speed between the lower limit set by parameter ω and upper limit set by parameter ξ. This is chosen randomly from a Gaussian random number generator.
	Contact with RET Ligand Expressing Cell	Domain	• For lymphotoxin signalling to occur, the bind between the two cells must be of sufficient strength. If the bind affinity is sufficient, we assume that cell signalling always occurs. • If contact is with a cell expressing RET ligand yet not an LTo, and a stable bind occurs, the cells will bind briefly but no signalling occurs
		Platform	• Whether LTin and LTo cells bind will be determined by a probability function. If a chosen probability is > parameter γ then a stable bind is formed
	Localised Movement Around LTo mediated by adhesion	Domain	• An LTin cell will remain in contact with an LTo cell if there is a sufficient expression level of adhesion factors • As expression level increases, the LTin cell is more likely to remain in contact • Though there may be sufficient expression level of adhesion factors, there is still a possibility that the LTin cell may move away from the LTo • Though the cell remains in contact with the LTo, LT Signalling and up-regulation of adhesion factors and chemokines only occurs on initial contact
		Platform	• LTin cell will remain in close contact with the LTo cell making small movements around it. When an LTin cell is held in direct contact to an LTo cell, the cell will remain in its current location. • Prolonged adhesion is decided through use of a probability function. Diagram in Figure 6 details how adhesion has been captured in the Platform Model.
	Other Assumptions	Domain	• LTin cells migrate into the tract throughout the whole period being modelled. • All LTin cells are the same size, 8μm
		Platform	• Through FACS staining we are aware of the number of LTin cells that should be present at E15.5 in development. A linear input rate is used to ensure this is reached. This rate remains constant throughout the simulated period • The environment is modelled as a 2D plane on which all movement and interactions occur (see environment in Platform Model). Should an LTin cell leave the left or right of the screen, this cell will be removed from the simulation.

Table 2: LTi Assumptions

Agent	State	Model	Assumptions
LTi	Random Movement on Tract Surface	Domain	• Cells move randomly until the level of chemokine expression in the vicinity is above a threshold
		Platform	• To ascertain chemokine level, the simulator will calculate the expression level in each 'gridsquare' around the cell (see Modelling Chemokines for more information). If none of these values is above φ, the cell moves randomly
	Response to Chemokine Level in Local Environment	Domain	• Three chemokines are known to play a part in the process - CXCL13, CCL19 & CCL21. However as an abstraction we will assume these can be modelled as a single chemokine (see Modelling Chemokines) • IL-7, throw stimulation of IL-7 receptor signalling might work in part through regulating chemokine receptor expression levels on LTi cells, has not be included in the model. The assumption will be made that there is always sufficient IL-7 present for chemokine receptor expression to be upregulated • There is always a small chance that the cell may not respond to the level of chemokine, although the expression level may be greater than φ.
		Platform	• Chemokine expression is modelled using an inverse sigmoid curve (see Modelling Chemokines). • As some stochasticity must remain, the chance that the cell will move in the direction of the strongest level is determined by probability function
	Contact with RET Ligand Expressing Cell	Domain	• For lymphotoxin signalling to occur, the bind between the two cells must be of sufficient strength. If the bind affinity is sufficient, we assume that cell signalling always occurs. • If contact is with a cell expressing RET ligand yet not an LTo, and a stable bind occurs, the cells will bind briefly but no signalling occurs
		Platform	• Whether LTi and LTo cells bind will be determined by a probability function. If a chosen probability is > parameter □□then a stable bind is formed
	Prolonged Surface Contact (Adhesion Effect)	Domain	• An LTi cell will remain in contact with an LTo cell if there is a sufficient expression level of adhesion factors • As expression level increases, the LTi cell is more likely to remain in contact • Though there may be sufficient expression level of adhesion factors, there is still a possibility that the LTi cell may move away from the LTo • Though the cell remains in contact with the LTo, LT Signalling and up-regulation of adhesion factors and chemokines only occurs on initial contact
		Platform	• The LTi cell would remain in close contact with the LTo cell making small movements around it. When an LTin cell is held in contact to an LTo cell, the cell will remain in its current location. • Prolonged adhesion is decided through use of a probability function. See Figure 6 which details how adhesion has been captured in the Platform Model (Modelling Adhesion)
	Other Assumptions	Domain	• LTi cells migrate into the tract throughout the whole simulated period • All LTi cells are the same size – 8μm
		Platform	• Through FACS staining we have determined the number of LTi cells that should be present in the mid-gut at E15.5 in development. A linear input rate is used to ensure this is reached. This rate remains constant throughout the simulated period • The environment is modelled as a 2D plane on which all movement and interactions occur (see Modelling the Environment in Platform Model). Should an LTin cell leave the left or right of the screen, this cell will be removed from the simulation.

Table 3: LTo Assumptions

Agent	State	Model	Assumptions
LTo	No Expression of RET Ligand	Domain	• Although we are aware that 20% of the intestine tract contains stromal cells, we assume only a percentage of these have the potential to become patches.
		Platform	• Where only a percentage of LTo cells are active, all are still placed on the intestine tract, but interactions only occur with LTo cells which have the potential to become patches (that express RET ligand).
	Expression of RET Ligand	Domain	• Cell will remain active throughout the time period, irrespective of whether the cell changes state or not
		Platform	• All LTo cells which express RET ligand have the potential to express adhesion factors and chemokines (thus form patches)
	Upregulation of Adhesion Molecules	Domain	• Adhesion molecules are up-regulated with every contact where the strength of the bind is sufficient (see Modelling Adhesion) • Up-regulation only occurs on initial contact with the cell – prolonged contact due to adhesion does not lead to further up-regulation • Cells in this state will divide after a set number of hours
		Platform	• Expression of adhesion factors does not degrade over time • With each stable contact, a counter representing adhesion factor expression is increased. This determines the strength of adhesion and probability the cell will remain in contact. See Modelling Adhesion.
	Upregulation of Chemokines	Domain	• Chemokines are up-regulated with each LTi/LTo contact where the strength of the bind is sufficient (see Modelling Chemokines) • Up-regulation only occurs on initial contact with the cell – prolonged contact due to adhesion does not lead to further up-regulation • Cells in this state will divide after a set number of hours
		Platform	• Chemokine expression does not degrade over time • With each stable contact, a counter representing chemokine expression is increased. This determines the distance over which the chemokine has an effect. See Modelling Chemokines.
	Mature LTo	Domain	
		Platform	• Both Adhesion molecules & Chemokines must have reached their peak of expression to reach this state
	Other Assumptions:	Domain	• It is assumed that other pathways, such as the NF-kB pathway, are always activated upon stable contact, and thus not explicitly modelled.