

CCR8-expressing cell with an EC<sub>50</sub> of between about 0.05 pM and about 50 pM, preferably between about 0.1 pM and about 10 pM; more preferably between about 0.3 pM and about 7 pM; and even more preferably between about 0.6 pM and about 3 pM. These EC<sub>50</sub> values are based on the CD16 cross-linking assay described in Example 17.

5           The direct killing of activated Tregs and patient tumor Tregs was demonstrated in Examples 19 and 20. In certain embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof mediates depletion of activated Tregs with an EC<sub>50</sub>, as measured by an apoptosis assay, of about 500 pM or lower; preferably about 100 pM or lower; preferably about 30 pM or lower; more preferably about 15 pM or lower; even more preferably  
10   about 5 pM or lower; or yet more preferably about 1 pM or lower. In certain preferred embodiments, the anti-hCCR8 mAb mediates depletion of the CCR8-expressing cell with an EC<sub>50</sub> of about 13 pM. In certain embodiments, the anti-hCCR8 mAb mediates depletion of the CCR8-expressing cell with an EC<sub>50</sub> of between about 1 pM and about 500 pM, preferably between about 5 pM and about 100 pM; and more preferably between  
15   about 10 pM and about 50 pM. These EC<sub>50</sub> values are based on the apoptosis assay described in Example 19.

#### *No Internalization of CCR8 by Anti-CCR8 mAbs*

Abs specific for certain cell surface receptors induce internalization of the receptor through receptor-mediated endocytosis which may be essential for targeted  
20   delivery of certain drugs, toxins, or enzymes for therapeutic applications. As shown in Figure 19, an anti-Inducible T cell Co-Stimulator (ICOS) mAb does not cause internalization of the ICOS receptor in the absence of a cross-linking Ab, but in the presence of the cross-linking Ab causes significant ICOS internalization. In contrast, an anti-CCR8 mAb causes no internalization of CCR8 either in the presence or absence of a  
25   cross-linking Ab. Internalization of CCR8 by an anti-CCR8 Ab would reduce its ability to mediate Treg depletion due to removal of the receptor from the Treg cell surface. Therefore, the lack of CCR8 internalization by anti-CCR8 Abs further validates the therapeutic potential of these Abs. CCR8 internalization may be measured by standard techniques used in the art, *e.g.*, the experimental protocol in Example 21.

30           Accordingly, this invention provides an anti-CCR8 mAb or antigen-binding portion thereof that, when bound to CCR8 on the surface of a cell, does not cause internalization of CCR8 either in the presence or absence of a cross-linking Ab. In certain preferred embodiments, the cell expressing CCR8 on the cell surface is a Treg.