Aoife Corcoran

1,25D is a steroid hormone that is responsible for the regulation of a number of biological processes such as its classical role in regulating calcium/phosphate homeostasis. In addition, $1,25D_3$ has been shown to have a number of non-classical roles including, anti-cancer activities such induction of cell differentiation in AML cells. Because of its anti-cancer abilities, $1,25D_3$ has become of therapeutic interest. However, the pharmacological doses required to induce a desirable biological effect lead to toxic side effects such as hypercalcemia. Therefore a number of studies are investigating the potential of 1,25D analogues and their therapeutic use. A number of analogues have since been designed with the intent to increase biological activity and reduce calcemic activities. Studies have suggested that $1,25D_2$ is less toxic in comparison to $1,25D_3$, therefore a number of research groups are currently working on designing and synthesizing analogues of $1,25D_2$. It is believed that only subtle changes introduced to the structure of 1,25D can lead to desirable biological side effects. For many years the focus of 1,25D analogues have been on analogues with modifications at one single location in the structure, designated single point modified (SPM) analogues. However, in recent years studies have investigated the therapeutic potential of analogues modified in two separate positions of the structure, designated DPM analogues.

In this study the biological activity of three separate series of $1,25D_2$ analogues are evaluated. In the first series of analogues the geometric isomers (PRI-1916 and PRI-1917) of the previously studied analogues PRI-1906 and PRI-1907 were evaluated in order to determine the significance of the orientation of the side chain. From this series of analogues it was found that by changing the orientation of the side chain, the overall activity of the analogue was also altered. The geometric isomer of PRI-1907 displayed lower activity, while the geometric isomer of PRI-1906 displayed slightly higher activity in comparison to the parental compund. In the second series of analogues the biological significance of the 5E,7E triene modification, unsaturation at C-22, and 24-epi was evaluated (PRI-1730, PRI-1731, PRI-1732, PRI-1733, and PRI-1734). However, these modifications led to a divergent series of analogues with lower VDR affinity and pro-differentiation activities in comparison to the parent hormones, $1,25D_3$ and $1,25D_2$. Interestingly by combining all three modifications to one of the analogues, the differentiation activity of PRI-1734 was completely disrupted. In addition PRI-1734 did not display any antagonist activity; however the structure may be of

interest as a starting point for possible antagonists. In the final series of experiments DPM analogues containing the side chain from PRI-1906 and PRI-1907 were investigated in combination with the *19-nor* modification resulting in PRI-5201 and PRI-5202. In vivo studies on mice found that the analogues had lower calcemic activities in comparison to $1,25D_3$. In addition these analogues had increased pro-differentiating activities as well as transcription enhancing activities. However the biological activity of the analogues did not correlate with their affinity to VDR.

In the second section the epigenetic mechanisms that may control expression of VDR were investigated. In addition cells that don't normally respond to $1,25D_3$ were treated with both $1,25D_3$ and epigenetic modulators to determine whether cells can undergo differentiation when combined with epigenetic modulators. KG1 cells treated with HDACIs and $1,25D_3$ were found to undergo cell differentiation, in addition levels of VDR expression were increased.