

IN THE HIGH COURT OF JUSTICE
BUSINESS AND PROPERTY COURTS OF ENGLAND AND WALES
INTELLECTUAL PROPERTY LIST (ChD)
PATENTS COURT

B E T W E E N:

(1) GILEAD SCIENCES INC.

(a company registered in the US State of Delaware)

(2) GILEAD SCIENCES LTD

Claimants

- and -

NUCANA PLC

Defendant

SECOND EXPERT REPORT OF MATTHIAS GÖTTE

I, MATTHIAS GÖTTE of 6-020K Katz Group Centre for Health Research, University of Alberta, Edmonton, Alberta, T6G 2E1, Canada **WILL SAY** as follows:

A. INTRODUCTION

1. I am the same Matthias Götte who provided a previous expert report on behalf of Gilead in this matter (my “**First Report**”). I adopt the definitions set out in my First Report.
2. I make this second report in reply to various points raised in the expert report of Professor Katherine Seley-Radtke, on behalf of NuCana. The fact that I make no

comment on any particular statements in that report should not be taken to mean that I agree with those statements.

3. I set out my understanding of my duty to the Court in paragraph 17 of my First Report and confirm that I have complied with that duty in making this second report.

B. THE SKILLED TEAM

4. In paragraph 21 of her report, Professor Seley-Radtke describes the Skilled Team as “*a team of individuals involved in the development of new nucleoside analogues with cytotoxic activity*”. Then in the same paragraph she states that “*the Skilled Team would have included a preclinical researcher with expertise in the identification and testing of compounds with cytotoxic activity*”. I am informed by B&B that part of NuCana’s case is that the technical contribution of the patents is either the provision of a novel class of phosphoramidate nucleotides with a not further defined “cytotoxic activity” or the provision of a novel class of phosphoramidate nucleotides with improved intracellular delivery.
5. As I stated in my First Report, nucleoside analogues had two well-described uses in 2003: as anti-cancer drugs and as antiviral drugs. It would have been reasonable for a team interested in the development of antiviral drugs to read the Patent and to consider potential antiviral effects and potential cytotoxic effects of the compounds (as cytotoxicity is an unwanted side-effect in antiviral compounds). In consequence a team interested in the development of antiviral drugs would be interested in finding compounds with good antiviral effects but which lack significant cytotoxic activity. In other words, and as I explained in my First Report, a key criterion for the skilled virologist is the selectivity index. The skilled virologist would certainly be identifying compounds with (unacceptable) cytotoxic activity for the purposes of removing them from further consideration as potential antiviral drugs. With regard to “improved intracellular delivery”, a team interested in the development of antiviral drugs would be interested in improved intracellular delivery only in the context of a nucleoside analogue which already demonstrated good antiviral effects. The skilled virologist would be interested in such an approach if there was evidence that it might be possible to improve on these pharmacological properties. This may not always be the case:

many approved HIV-1 nucleoside reverse transcriptase inhibitors (NRTIs) were approved in the absence of a prodrug strategy.

C. COMMON GENERAL KNOWLEDGE

6. In section C.1 of her report, Professor Seley-Radtke describes the drug discovery process, depicted as a “funnel” which narrows from drug discovery at the top to regulatory phase at the bottom, with (although she does not say this) approved medicines emerging from the very bottom of the funnel. Professor Seley-Radtke describes the pre-clinical phase as having three stages:

“(i) identifying compounds with the desired activity in vitro; (ii) refining these compounds to a smaller sub-set with the desired potency; and (iii) further refining these selected molecules into an even smaller sub-set that not only shows the desired potency, but satisfy other preferred criteria (such as being able to cross cell membranes, being orally bioavailable, not exhibiting unwanted side effects and not being metabolised into inactive forms by the body, among other drug-like properties).”

7. From the point of view of the Skilled Virologist, cytotoxic activity which would be the (stage (i)) “*desired activity*” in an anti-cancer compound would be an (stage (iii)) “*unwanted side effect*” in an anti-viral. For an antiviral, inhibition of virus replication would be the desired activity to be assessed in stage (i) and desired antiviral potency would then be refined in stage (ii). Any problematic cytotoxicity would be assessed in parallel with antiviral activity as an assessment of the selectivity index.

8. At paragraph 30 of Professor Seley-Radtke’s report she states:

“There is therefore a balancing act between increasing potency and achieving other properties in order to maximise the potential of a molecule as a drug candidate. The Skilled Team at the Priority Date would have been aware of this and of the value of classes of compounds that already displayed potency and other “drug-like” qualities.”

9. This balancing act is clear for a potential drug candidate in a particular therapeutic field – i.e., anti-cancer or anti-viral. However, the balancing act is dependent on the field in

question. Cytotoxicity appears on one side of the balance or the other depending on the proposed use.

10. This is particularly an issue in the context of rational drug design and SAR as mentioned in paragraph 29 of Professor Seley-Radtke's report.
11. As I will describe in more detail below, antiviral activity is highly dependent on the particular virus and the particular nucleoside analogue being studied. It is unrealistic to discuss SAR and rational drug design and seek to predict antiviral efficacy across the entire scope of viruses – both DNA and RNA viruses – and across a wide class of different nucleoside analogues. As I stated a number of times in my First Report, this field was in 2003 largely empirical. Small changes to a molecule can have a significant impact on activity.
12. To illustrate this, I refer to paragraph 33 of Professor Seley-Radtke's report in which she cites Perkins (1962) as showing the early work on nucleoside analogues in medicinal chemistry. Perkins is a paper published in Nature which describes several iodine-substituted deoxyribonucleotides including 5-iodo-2'-deoxycytidine (called 'ICDR' in the paper), 5-iodo-2'-deoxyuridine (IUDR) and 5-iodo-uridine (IUR) and studies their effect in herpes simplex infections in rabbits. The paper states on page 985:
- L tab. 143 vol 3**

“Both IUDR and ICDR have been shown to inhibit the biosynthesis of deoxyribonucleic acid (DNA) and, in addition, to be incorporated as IUDR 5'-phosphate into DNA, in place of thymidine 5'-phosphate. This communication confirms the efficacy of IUDR in the treatment of an infection of rabbit eyes with a DNA-virus, namely, herpes simplex, and describes a similar anti-viral activity for ICDR...”

13. However on page 986, it is stated that :

“In addition, it was shown that 5-iodo-uridine (IUR), the ribonucleoside of 5-iodo-uracil, a compound which undergoes metabolic degradation in a manner similar to that of IUDR, but exerts no inhibitory effect on the biosynthesis of DNA, is without anti-viral activity.”

14. Even a change which on its face is small – swapping a 2'-H atom (deoxyuridine) in the sugar for a 2'-OH group (uridine) caused complete loss of antiviral activity. By 2003 it was known that compounds with a 2'-H atom would be accepted by DNA polymerases or reverse transcriptase whereas compounds with 2'-OH would be accepted by RNA polymerases. Therefore the 2' position in a nucleoside analogue is highly significant in an antiviral context as it differentiates between DNA viruses or retroviruses and RNA viruses, respectively, as potential targets.

D. NUCLEOSIDE ANALOGUES

15. In paragraph 36 of Professor Seley-Radtke's report she describes, in general terms, the mechanisms of action of the active form of nucleoside analogues.
16. There are some points from the perspective of the skilled virologist that I would like to make about her discussion of mechanisms of action in paragraph 36 and in the subsequent sections C.2.1 and C.2.2 in terms of antiviral nucleoside analogues.

Paragraphs 36(a) & (b)

17. In paragraphs 36(a) and (b) Professor Seley-Radtke describes the first two potential mechanisms of action for nucleoside analogues:

“(a) incorporation of the active nucleotide into a nucleic acid chain by enzymes called polymerases (including reverse transcriptases) in preference to naturally occurring nucleosides;

(b) preventing enzymes from working properly (for example, those involved in nucleic acid synthesis, such as polymerases) which may lead to chain termination”

18. From the point of view of the skilled virologist these two mechanisms would be more suitably described together as competition of the nucleotide analogue with its the natural counterpart, binding of the nucleotide analogue to a polymerase enzyme, incorporation of the nucleotide into the nucleic acid chain followed by chain termination or potentially other mechanisms of inhibition. This is discussed in more detail below.
19. The putative mechanism of action for a nucleoside analogue would not be discernible from the structure of the relevant molecule perhaps with the exception of obligate chain

terminators, as I describe below. A particular nucleoside analogue must be investigated experimentally to establish what effect it has and the mechanism by which that is achieved.

Chain Terminators

20. It is worth explaining the concepts of obligate and non-obligate chain termination. Chain termination is the key mechanism of action where a nucleotide analogue lacks a 3'-OH group. It is a clearly defined process and I described it in paragraph 53 of my First Report. DNA or RNA chain extension cannot occur in the absence of a 3'-OH group that is essential for the nucleophilic attack on the alpha-phosphate of an incoming nucleotide. This is why nucleoside analogues lacking a 3'-OH group are referred to as "obligate chain terminators". On the other hand, where the nucleoside analogue does have a 3'-OH group, then there is the possibility of continuation of DNA/RNA chain synthesis. Inhibition is not a given because the nucleophilic attack on the alpha-phosphate of an incoming nucleotide is theoretically possible in the presence of a 3'-OH group or a structural equivalent. Such molecules are "non-obligate chain terminators"¹ in that a particular nucleoside analogue may cause chain termination but a different nucleoside analogue may not.
- L tab.152 vol 3
21. It is therefore relevant to note that Formula I and III in the Patent and Divisional each contain a mandatory 3'-OH group. This will mean that none of the compounds in the class will be obligate chain terminators. Leaving aside the requirements for polymerase binding and incorporation, neither of which are certain to occur, chain termination might ultimately result from use of a particular nucleoside analogue within Formula I and III, or it might not. Whether a nucleoside analogue which contains a 3'-OH group will function as a chain terminator would not be self-evident from merely looking at the structure of the molecule. It would need to be tested in order to determine whether it acts in this way.

Paragraph 36(c)

22. In paragraph 36(c), as her third mechanism of action, Professor Seley-Radtke refers to:

"preventing naturally occurring nucleotides from being metabolized or synthesized, for example, by inhibiting enzymes involved in other biological processes, such as thymidylate synthase (TS) and ribonucleotide reductase (RNR)"

¹ See Reardon (1989) J Biol Chem 264(32), 19039-19044

23. Mechanisms such as TS and RNR inhibition are not to my knowledge desired targets for antiviral nucleoside analogues. TS and RNR are host cell factors and targeting these can be associated with cellular toxicities that may affect virus replication only indirectly.

E. USE AS ANTIVIRALS

24. In section C.2.1, Professor Seley-Radtke comments on each of the various nucleoside analogue antiviral drugs which had been approved by 2003 to treat HSV, VZV, HIV, HBV, HCV and CMV.
25. The mechanism of action of nucleoside analogue antiviral drugs varies. Other than in the case of an obligate chain terminator, discussed above, incorporation of an active (triphosphate) nucleotide into a nucleic acid chain in preference to naturally occurring nucleotides does not inevitably lead to inhibition of nucleic acid synthesis. There must be a mechanism that inhibits DNA or RNA synthesis afterwards.

Herpesvirus drugs

26. In paragraphs 40 to 55 of her report, Professor Seley-Radtke describes the approved drugs for treating the various Herpesvirus infections. With the exception of acyclovir (described at paragraph 44 of her report), which is an obligate chain terminator, the remaining drugs have a 3'-OH group (or a structural equivalent of a 3'-OH group) meaning that these would be considered as non-obligate chain terminators and their mechanism of action can differ.

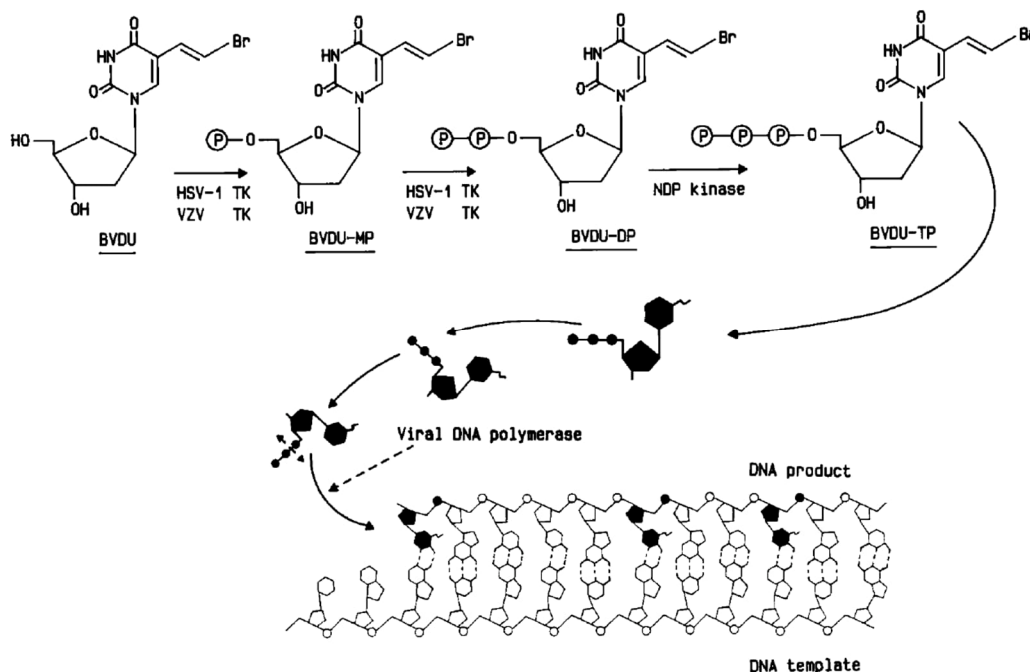
Brivudine

27. One of the drugs mentioned by Professor Seley-Radtke is Brivudine (BromovinyldeoxyUridine) (BVdU) which I referred to in my First Report in the context of the Patent. She describes the mechanism of action for BVdU as follows:

“The active triphosphate form works by targeting viral DNA polymerase to compete with deoxythymidine triphosphate (dTTP) and thereby inhibits viral DNA synthesis”

28. In fact, the mechanism of action of BVdU would be more accurately described as *“incorporation of a nucleotide analogue into a growing DNA/RNA chain, without proceeding to chain termination”*.

29. The mechanism of action of BVdU is described in a key 1997 review article by DeClercq.² It is illustrated below in Figure 3 taken from that paper, reproduced here.



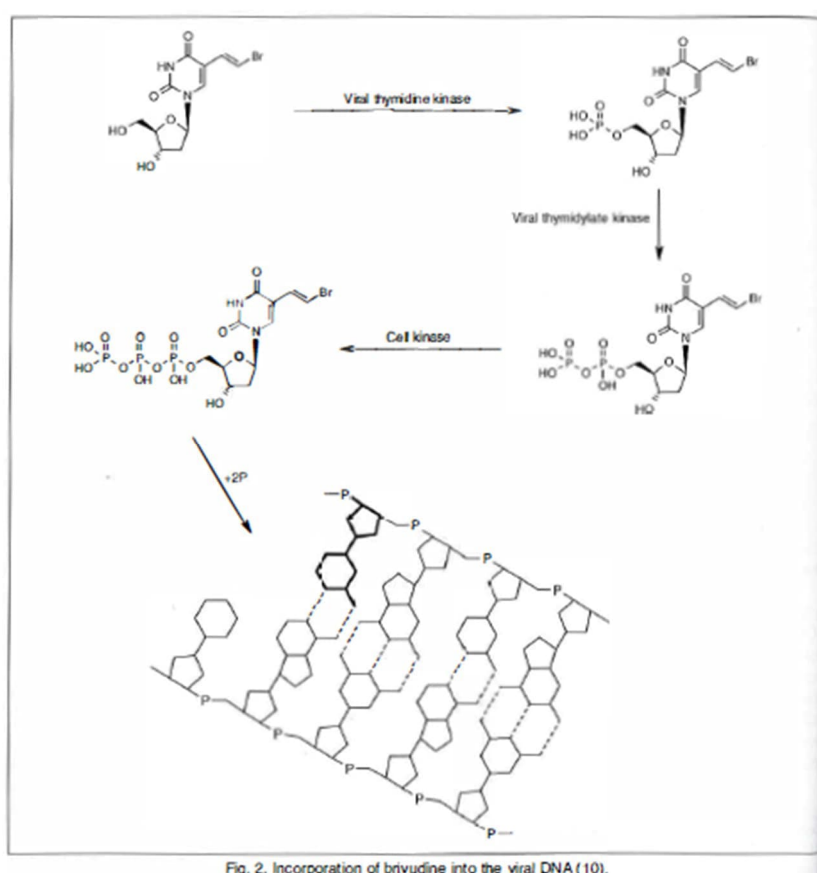
30. The figure is explained in the body of the paper on page 676:

“The mechanism of action of BVdU (Fig. 3) depends on a specific phosphorylation by the virus-encoded TK, i.e., the TKs induced by HSV-1 and VZV, which convert the compound to its 5'-monophosphate (BVdU-MP) and 5'-diphosphate (BVdU-DP). Upon further phosphorylation by cellular kinase(s), i.e., nucleoside 5'-diphosphate (NDP) kinase, BVdU 5'-triphosphate (BVdU-TP) can act in a dual fashion with the viral DNA polymerase: (i) as a competitive inhibitor with respect to the natural substrate (dTTP) or (ii) as an alternative substrate, which would then allow BVdU-TP to be incorporated (as BVdU-MP) into the DNA chain. This incorporation may, in turn, affect both the stability and the functioning of the DNA. In fact, a close correlation has been found between the incorporation of BVdU into HSV-1 DNA, viral infectivity, and DNA integrity.”

31. The text refers to phosphorylation of BVdU by viral encoded TK (thymidine kinase) enzymes “which convert the compound to its 5'-monophosphate (BVdU-MP) and 5'-

² DeClercq (1997) Clin. Microbiol. Rev. 10(4): 674-693

diphosphate (BVdU-DP)". The 5'-diphosphate is then acted upon by a cellular kinase to convert the 5'-diphosphate to the 5'-triphosphate. This refers to a very interesting feature of BVdU and why it is an effective inhibitor of herpes viruses such as VZV and HSV-1. The genomes of HSV-1 and VZV encode the genes for viral TK enzymes which, following infection of a host cell, are expressed in the cell. These viral TK enzymes must be present in order to produce the 5'-monophosphate and 5'-diphosphate. The Rabasseda 2003 paper cited by Professor Seley-Radtke in paragraph 52 of her report also refer to these findings. Figure 2 on page 360 of the Rabasseda paper illustrates this.



32. Also, the text on page 361 states:

"Brivudine is a deoxythymidine nucleoside analog that is phosphorylated sequentially by viral thymidine kinase, viral thymidylate kinase and cellular kinases and then incorporated into the viral DNA by means of the polymerase (Fig. 2). Brivudine cannot be phosphorylated by eukariote cell nucleoside kinases, and so it is highly selective for viral DNA."

33. This interesting feature of BVdU means that it could not be efficiently converted to the 5'-active triphosphate unless the cell was infected or otherwise expressed viral TK enzymes. For example, in paragraph 54 of her report, Professor Seley-Radtke describes exactly this. **L tab.13 vol 1** She refers to the Balzarini 1989 paper in which BVdU was investigated for anti-tumour activity in a tumour cell line. In order to overcome the BVdU TK phosphorylation issue, the tumour cell line in question (FM3A) was "*genetically engineered such that they contain and express HSV-1 or HSV-2 dThd kinase gene*". Without viral TK enzyme in the tumour cell, BVdU could not be efficiently converted to the 5'-triphosphate.
34. Returning to the DeClercq 1997 paper, the text (see paragraph 30 above) then refers to two modes of activity: (i) competitive inhibition with the natural nucleoside (dTTP) or (ii) acting as an alternative substrate. Competitive inhibition with respect to dTTP means that the BVdU-TP competes with the dTTP to bind to the polymerase enzyme but without necessarily resulting in incorporation of the nucleotide into the DNA chain.³ **L tab.48 vol 1**
35. Competitive inhibition in the absence of subsequent nucleotide incorporation, and, in turn, without causing chain termination, was a possible mode of inhibition which was quite vaguely discussed in the literature. Such inhibition could theoretically occur. Enzyme kinetics as described by Karen Anderson (Yale University) distinguish between the efficiency of nucleotide binding and the efficiency of the subsequent chemical step that causes incorporation.⁴ Again, whether a nucleotide analogue will function as a competitive inhibitor without being incorporated and act as a chain terminator would not be self-evident from merely looking at the structure of the molecule. It would need to be tested; ideally in gel-based assays that show the products of the reaction. **L tab.7 vol 1**
36. The reference in the above passage in DeClercq 1997 to acting as "*an alternative substrate*" means that the BVdU-TP is doing everything that the natural substrate would do – it binds to the viral polymerase, it becomes incorporated into the DNA chain and DNA synthesis then continues not necessarily with any form of inhibition. As shown in the figure from DeClercq 1997 in paragraph 29 above, the BVdU nucleotides (shown shaded black) have been incorporated into the DNA chain but DNA synthesis has continued uninterrupted. A specific mechanism of action is not evident.

³ I mentioned this mechanism as a possibility in my First Report (on page 15, at footnote 10)

⁴ Anderson (2002) Biochim. Biophys. Acta, 1587, 296-299

37. The unusual and poorly understood mechanisms of action of BVdU were an additional reason why information about the activity of BVdU or BVdU analogues could not be used to draw any conclusions about the activity of other nucleoside analogues.

HIV drugs

38. In paragraphs 56 to 69 of Professor Seley-Radtke's report she lists the various HIV drugs which had been approved by 2003.

39. The first point to make is that all of these drugs lack a 3'OH group and are therefore all obligate chain terminators. These HIV drugs do not have any direct relevance *per se* to the class of 3'-OH containing, non-obligate chain terminators described in the Patent because they operate by different mechanisms.

40. The mechanism of action of obligate chain terminators is well-defined. However, the differences in levels of activity between different obligate chain terminators can involve complicated issues associated with nucleotide binding and the efficiency of the chemical step as described above (Anderson (2002)).

L tab.7 vol 1

41. Two of the HIV drugs listed by Professor Seley-Radtke, 3TC and d4T, are discussed in the Patent. I discussed d4T in my First Report (paragraphs 85 to 89) in the context of the Balzarini (1996) paper which is cited in paragraph [0008] of the Patent.

L tab.17 vol 1

42. In my First Report, I stated that the patent "*appears to extrapolate from the antiviral results shown in the Balzarini paper and claims that the same phosphoramidate approach is applicable to cancer therapy... However, the skilled virologist would know that a virologist and an oncologist would have opposing views on the potential usefulness of cytotoxic compounds... Therefore, to the skilled virologist there would be no basis upon which an extrapolation can be made that a compound which is useful as an antiviral will be useful as an anti-cancer agent. That makes no sense at all.*"

43. As explained above, even well-studied obligate chain-terminators such as the aforementioned HIV drugs can show complex different kinetic properties that translate in differences in efficiency of inhibition. Given the relative lack of knowledge about mechanisms of action of the non-obligate chain terminators, seeking to predict any activity

for an entire class of nucleoside analogues would not have made sense. In a class where antiviral activity is not a given and must be tested each time and where even a known active antiviral compound could have an uncertain mechanism of action, I do not believe that the skilled team could have made legitimate predictions as to likely activity based on structural changes from a nucleoside analogue inhibitor with known activity. As I have stated previously, in the field of nucleoside analogue inhibitors even small structural changes can have significant effects.

STATEMENT OF TRUTH

I confirm that I have made clear which facts and matters referred to in this report are within my own knowledge and which are not. Those that are within my own knowledge, I confirm to be true. The opinions I have expressed represent my true and complete professional opinions on the matters to which they refer.

I understand that proceedings for contempt of court may be brought against anyone who makes, or causes to be made, a false statement in a document verified by a statement of truth without an honest belief in its truth.

Signed: 
Professor Matthias Götte

Dated: 6 December 2022