Early phase clinical drug development for HPV-induced disorders: novel tools and treatments

Rijsbergen, M.

Chapter 7

SUMMARY AND DISCUSSION

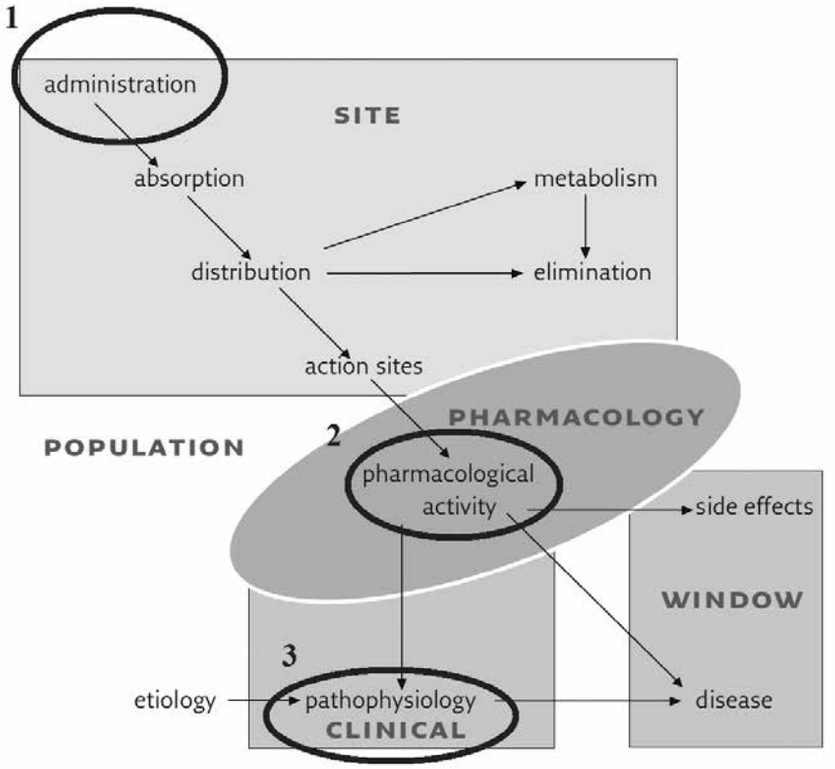
The scope of this thesis was to develop and implement new methods for the monitoring of HPV-induced disease and to elucidate novel pharmacological interventions for these disorders. The overall disease burden of HPV infections is high. As responses to current treatments are poor and recurrence rates are high, there is a strong medical need for new, effective drugs that eliminates the virus with an acceptable side effect profile. A rational, question-based development approach that integrates the investigation of the pharmacological effects in early phase drug development, will obviously be consuming less time and resources. This approach was described in 2003 in an attempt to efficiently investigate the pharmacological effects in early phase drug development.[1] In this thesis, 3 main questions of this approach were applied to clinical drug development in HPV-induced diseases as shown in Figure 1:

1. Does the drug get to the site of action, i.e. is it administrated as prescribed?
2. Does the compound cause its intended pharmacological effect?
3. Does the compound have beneficial effects on the disease?

It is essential to utilize the most appropriate methodology to answer these questions. Special attention was therefore given to the development of new methodological tools to monitor the course of HPV-related diseases in clinical trials, as well as the exploration of successful biomarkers of viral load after HPV infections. In this thesis, studies that address different aspects of early clinical phase drug development in three different HPV-related diseases are presented. This thesis is divided into two parts: section 1 describes the development and application of novel tools in clinical drug development and section 2 focuses on early phase clinical studies examining safety, tolerability, pharmacodynamic and efficacy parameters of new topical compounds with high potential for the treatment of HPV-induced diseases.

The current chapter provides a discussion of the results presented in this thesis and concerns: 1) the implementation of tools and biomarkers in early clinical trials in patients with HPV-induced disease, 2) the investigation of potential novel medical treatments of HPV-induced diseases and 3) the implications of these findings for future clinical drug development in HPV-induced diseases.

Figure 1. Schematic representation of the 3 main questions during the course of action of a drug. The first question enhances different aspects regarding reaching the site of action. We focused on one of these aspects; the administration of the drug (1) and developed an e-diary to measure the medication adherence of the patients in Chapter 2. The second question entails demonstration of the mechanism of action of the drug by showing its pharmacological activity (2) and is investigated with the use of viral load measurements in chapter 4, 5 and 6. The third question refers to the clinical efficacy which is investigated using three-dimensional photography in chapter 3. This figure is adapted from S. de Visser 2003.[1]



THE DEVELOPMENT AND IMPLEMENTATION OF TOOLS AND BIOMARKERS IN EARLY CLINICAL TRIALS IN PATIENTS WITH HPV-INDUCED DISEASE

1. DOES THE DRUG GET TO THE SITE OF ACTION?

In order to get to an answer of the first question of the question-based approach we developed an e-diary to investigate the administration of the drug by treatment adherence. Most compounds for HPV-induced diseases are topical drugs administered directly on the lesion by the patient himself at home. Importantly, medication adherence to long-term therapy is approximately 50% and adherence to topical drugs is even poorer than oral treatments.[2] **Chapter 2** described the development and implementation of the e-diary for the monitoring of treatment adherence and patient-reported outcomes in dermatological clinical trials to overcome the low adherence. The e-diary showed to be an excellent method to measure treatment administration as shown by the high treatment adherence rate (i.e. actual administrations divided by the expected administrations) of 98% (median; range 97-99%). E-diary adherence (i.e. actual entries divided by the expected entries) was also high with a median of 93% (range 87-97%) of photos capturing the applied drug. We hypothesized that this high adherence could have been the result of the designated reminder function of the e-diary to motivate patients to apply the drug on time. User acceptability of the e-diary was rated high by the patients as the e-diary was rated good to excellent by 89% of the patients and the user-friendliness was experienced as being good to excellent by 94% of the patients. Monitoring patient-reported outcomes by filling in daily symptom scores provided good insights in the disease burden. Patients filled in the itch and pain score with a median adherence of 89% (range 87-96%) and 94% (range 87-96%), respectively.

We can conclude that the e-diary seems a good tool to measure that the drug was applied on the lesions. Obviously it is not synonymous that the drug which is applied on the lesion also penetrates the skin or lesion and eventually accessing virally infected cells. However, animal models and in vitro experiments using human donor skin showed that the drugs investigated in this thesis do sufficiently penetrate the skin.[3,4] Also, the HPV-induced diseases under investigation in this thesis are all restricted to cells above the basal layer, i.e. the epidermis. This means that no transdermal drug delivery to the systemic circulation is necessary and even not desirable in case of digoxin for instance. The first question of the question-based drug development cannot be fully answered based on this research and as is shown in Figure 1 the first question entails several aspects, i.e. administration, absorption, distribution. Nevertheless, it is shown that the medication adherence is very high, implicating that the drug is administrated on the lesions and based on the preclinical studies it seems very likely that the drugs reached the sites of action.

2. DOES THE COMPOUND CAUSE ITS INTENDED PHARMACOLOGICAL EFFECT?

The second question was investigated by the measurement of the pharmacological activity of the compound. The use of a biomarker can be helpful in the early phase of clinical drug development, as it can be used as a quantitative indicator of a biological process.[5] Multiple biomarkers have already been tested for the prediction of treatment success and the prognosis in patients with HPV-induced diseases.[6–9] A useful biomarker 1) is easily applicable in clinical practice, 2) is reproducibly measurable over time, and 3) has a plausible relation between the biomarker, the expected pharmacological effects and the pathogenesis of the disease. While the drugs of concern in this thesis are hypothesized to have anti-viral effects, we choose to measure viral load of the lesions. Previous research indicated that viral load is positively related to the severity of the disease; an increase of viral load in biopsies and cytological samples of cervical lesions can predict the progression of the disease.[10,11] The gold standard to determine viral load is in biopsy samples, but these are invasive, remove (part of) the lesion and can only be performed for a limited number of times. Viral load can also be measured by taking a swab from the lesion, by rubbing the surface 5 consecutive times with a pre-wetted cotton-tipped stick. It was already shown in 2011 that swabs of cutaneous warts (CW) can reliably identify the HPV genotype.[12] In 2013, a study on genital lesions showed a high concordance between HPV genotyping by biopsy and swab in penile HSIL but only low to moderate concordance in AGW.[13] Concordance of viral load measurements in swabs and biopsy samples has never been studied in HPV-induced diseases. It is also unknown whether the viral load measured with swabs can be used to evaluate HPV-induced diseases over time, i.e. during treatment or follow-up. HPV genotyping and the determination of viral load of the lesions in HPV-induced diseases is of profound importance for the prediction of the drug efficacy and therefore may serve as a biomarker

during clinical drug development.[14] We implemented swabs to measure viral load in HPV-induced lesions over time in the trials described in **Chapter 4, 5** and **6**. Although it was found that viral load in swabs was highly variable per patient we detected significant differences in viral load over time between the different treatment groups. In Chapter 4 we evaluated viral load in biopsies and swabs of CW and found a significant correlation between both methods. Also, there was a significant correlation between the wart size reduction and the reduction in HPV load. Unfortunately, in AGW and vulvar HSIL it was not possible to compare swabs with biopsies because other laboratory techniques were used. Taking a swab is a noninvasive procedure that can be performed in the same lesion over time, but the procedure is also sensitive and has to be performed reproducibly and accurately, e.g. rubbing harder of more often could result in the collection of more viral cells. Therefore, it is important that the swab procedure is standardized and that all investigators use the same procedure. The implementation of this biomarker clarified whether the hypothesized working mechanism, i.e. anti-viral activity of the compounds, was applicable. Taken together, the measurement of viral load by taking swabs appears to be a good method to test pharmacological effects, i.e. antiviral activity, on HPV-induced lesions. With this knowledge, indications showing no anti-viral activity should not be further investigated and drug development for these specific indications should be discontinued. This approach helps saving time and resources by the early prediction of pharmacological activity and will assist in efficient drug development.

3. DOES THE COMPOUND HAVE BENEFICIAL EFFECTS ON THE DISEASE?

To determine the efficacy of a topical drug on HPV-induced lesions, it is important to frequently and precisely assess the lesions over time. For clinicians, it is often difficult to define the precise location and margins of lesions, especially in vulvar HSIL. Biomarkers can help visualizing the lesion for early detection and follow-up purposes.[15] Three-dimensional (3D) photography appears to be a good candidate biomarker to obtain comprehensive insight into the dimensions of lesions. The use of three-dimensional photography is already widely integrated into plastic surgery and anthropometry practice, but not yet applied in early phase clinical trials on drug development in HPV-induced diseases.[16–18] In clinical trials based on drug development, small changes in lesion morphology or dimensions might already predict a treatment response. We hypothesize that the 3D camera seems to be an optimal candidate biomarker of HPV-induced lesions. Therefore, the use of 3D photography was validated and investigated in **Chapter 3**. Three-dimensional photography with this specific camera system had an excellent accuracy and reproducibility. We also found a good to excellent agreement between different raters of the 3D photographs of the HPV-induced lesions. Caliper measurements of the dimensions of HPV-induced lesions are gold standard. We compared caliper and 3D measurements and found acceptable differences for the diameter of AGW, vulvar HSIL and CW and for the height of CW. Importantly, a difference between caliper and 3D measurements of the height of AGW lesions was found, probably because it was complicated to accurately measure height with a caliper of these lesions in the genital area. We speculate that 3D photography is a more reliable method than caliper-based measurements although hard evidence remains to be obtained. By using 3D photography, one might be able to determine the efficacy of a drug in an early stage of drug development. Moreover, 3D photography serves as an excellent method to clinically visualize the HPV-induced lesions as it is accurate and precise and enables researchers to compare different time points at once. The 3D camera might also be useful for the measurement of lesion surface and volume, which enables an adequate prediction of drug efficacy. We were not able to validate surface and volume measurements by the 3D camera in AGW, vulvar HSIL and CW, as these could not be measured with a caliper due to the asymmetrical and irregular shape of these lesions. Nevertheless, 3D photography seems a more suitable and versatile method to measure these lesions.

THE INVESTIGATION OF POTENTIAL NOVEL MEDICAL TREATMENTS OF HPV-INDUCED DISEASES

In the second section of this thesis two potential novel topical treatments for different HPV-induced diseases were examined: I) omiganan and II) ionic contra-viral therapy (ICVT). Omiganan is an antimicrobial peptide with immunomodulatory and anti-viral properties and was investigated in patients with AGW and vulvar HSIL as described in **Chapter 4**. Omiganan showed to be safe for both indications as there were no serious adverse events and all adverse events were of mild intensity and self-limiting. Omiganan significantly reduced viral load in AGW after 12 weeks of treatment once daily, but no clinical efficacy was shown. In vulvar HSIL, omiganan did not reduce the viral load

after 12 weeks of treatment and neither showed any clinical efficacy. ICVT is comprised of digoxin and furosemide and inhibits the potassium influx on which DNA viruses rely for replication. Safety and efficacy and in patients with CW was investigated in **Chapter 5**. ICVT was shown to be safe in patients with CW. ICVT treatment once daily for 6 weeks significantly reduced viral load and size of the CW. **Chapter 6** describes a clinical trial with ICVT in patients with AGW which showed that ICVT was well tolerated as there were no clinically relevant safety findings and no serious adverse events. Contrary to the findings in CW, ICVT in AGW patients did not show any pharmacological activity nor clinical efficacy.

DIFFERENCE IN PHARMACOLOGICAL ACTIVITY BETWEEN COMPOUNDS

It is interesting that viral load measurements showed that the compounds had varying pharmacological efficacy on the different HPV-induced lesions. Omiganan reduced viral load in AGW patients, but did not show any pharmacological activity in vulvar HSIL. ICVT showed to reduce viral load and wart size in CW, but showed no pharmacological activity in AGW. There are multiple hypotheses for these varying pharmacological effects in HPV-induced diseases.

HPV types are divided in different groups based on the alignment of the viral DNA. The HPV-induced diseases in this thesis are caused by HPV types from the Alpha genus type. Cutaneous warts are caused by low risk types HPV2, 27 and 57, AGW mostly by low risk type HPV6, while vulvar HSIL is mostly caused by the high risk type HPV16. The phylogenetic tree of HPV (see Figure 3 in the introduction of this thesis) shows that these types are evolutionary different.[19] Previous research indicated that the HPV type can predict treatment response in patients with CW.[20] It was found that HPV2 and HPV27 were associated with a limited response to the treatment of plantar warts with monochloroacetic acid or the combination of cryotherapy and salicylic acid. HPV1 can be a predictor of the response to the treatment of plantar warts with the combination of cryotherapy and salicylic acid. It is plausible that the difference in treatment response was caused by the difference in the causative HPV type.

We tested omiganan in AGW and vulvar HSIL. In AGW, treatment with omiganan reduced viral load, while in vulvar HSIL no effect was observed. AGW is a benign lesion caused by low risk HPV types, whereas vulvar HSIL is a premalignant lesion causes by high risk HPV types and can progress to vulvar cancer. Analyses of complete genome sequences have shown that HPV16 is more diverse with four variant lineages, compared to two variant lineages for HPV6.[21,22] These differences and their oncogenic properties may point towards the hypothesis that omiganan can interfere with the low-risk HPV type 6 and 11, but may not be effective for high-risk HPV type 16. An explanation might be that high risk HPV types cause integration of the viral DNA into the human genome and overexpression of the E6 and E7 oncoproteins, which are not amenable to omiganan treatment.

The location and lesion type might also be of importance when predicting treatment response, e.g. penetration of drug might be easier on the mucosa instead of on a cutaneous wart.

THE IMPLICATIONS OF THESE FINDINGS FOR FUTURE CLINICAL DRUG DEVELOPMENT IN HPV-INDUCED DISEASES

The question-based drug development approach is useful for the design and conduct of (early phase) clinical trials in HPV-induced diseases. As shown in this thesis, the e-diary is an adequate tool to measure treatment adherence and patient-reported outcomes. In addition to that, the e-diary could also stimulate timely use of medication. In future clinical trials, patient-reported outcomes need further investigation. Also, the e-diary might be highly valuable to adjust the treatment based on patient-reported outcomes in clinical practice. Integration of e-diaries in clinical practice is gaining increased attention, for example in COPD patients where electronic questionnaires are developed to predict symptom-defined exacerbations.[23]

Viral load measurement by using swabs is a suitable biomarker for the prediction of the anti-viral pharmacological efficacy of the drug. Viral load in swabs and biopsies had a good correlation in CW patients and therefore the swabs can replace the biopsies for the measurement of viral load in CW. The comparison of the viral load of swabs and biopsies in AGW and vulvar HSIL needs to be further investigated in future studies. For future implementation of 3D photography, validation of surface and volume measurements is warranted and another system might be necessary for the irregular shapes of the genital area.

We encountered some difficulties with the precise measurement of the vulvar HSIL dimensions with both caliper and 3D photography. Vulvar HSIL

is associated with a highly variable appearance and most of the time lesions have irregular and blurred borders and are difficult to recognize. In future drug development initiatives it would therefore be ideal to improve the visualization of these HPV-induced lesions, for example with a specific fluorescent ligand for HPV-induced lesions. Fluorescence imaging has well-known advantages in intraoperative settings. Particularly near-infrared (NIR) fluorescence seems interesting to investigate HPV-induced lesions, as its light increases tissue penetration depth up to 1 cm. A fluorescent agent can either be nonspecific or targeted at HPV positive cells for example. NIR fluorescence has already been widely investigated in cancer surgery for other indications, such as ovarian and colorectal cancer.[24,25] Imaging using NIR fluorescence should be further investigated in HPV-induced diseases and will hopefully facilitate the measurement of vulvar HSIL and other lesions in early stage clinical research. In addition, NIR fluorescence can also be valuable in clinical practice for lesion diagnosis and visualization during operations, to ensure total lesion removal.

In conclusion, in this thesis we implemented the question-based drug development approach in clinical trials in HPV-induced diseases. This led to the implementation of 1) the e-diary to confirm whether the drug has reached the site of action, 2) viral load measurement by swabs to determine the pharmacological activity of the compound and 3) three-dimensional photography to investigate the efficacy of the compound. Together, the described tools and biomarkers might be of high value for a more efficient drug development in HPV-induced diseases. Also, four clinical trials were performed with the topical drug omiganan and ICVT in patients with different HPV-induced diseases. These trials showed that both drug have different pharmacological and clinical efficacy depending on the HPV-induced disease.

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