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Topical Imiquimod as Primary Therapy Prior to Mohs Surgery for Penile Intraepithelial Neoplasia

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TOPICAL IMIQUIMOD AS PRIMARY THERAPY PRIOR TO MOHS SURGERY FOR PENILE INTRAEPITHELIAL NEOPLASIA

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Abstract

Penile intraepithelial neoplasia is a rare malignant lesion commonly caused by the human papillomavirus or associated with lichen sclerosus. The definitive treatment for these indolent lesions is surgery, however topical treatments are often first line as an organ-preserving strategy. Currently, the evidence for topical therapies is based on literature reviews and case studies, with no randomized trials to best guide treatment. In this study, we will conduct a double-blind, randomized placebo-controlled trial to determine whether the use of imiquimod 5% cream as a primary therapy will have higher complete response rates in penile intraepithelial neoplasia compared to placebo cream prior to definitive Mohs micrographic surgery. This study would provide robust evidence for the use of imiquimod as a monotherapy, as well as additional data on recurrence rates and the efficacy of Mohs micrographic surgery.

Chapter 1: Introduction

1.1 Background

Penile intraepithelial neoplasia (PeIN) is defined histologically as squamous cell carcinoma *in situ*, and is a precursor lesion to its invasive counterpart, penile cancer.[1] Other names for these lesions include penile squamous cell carcinoma *in situ*, Bowen's disease (BD), Bowenoid papulosis (BP), and, when localized to the glans penis, erythroplasia of Queyrat (EQ). PeIN can be further divided into two subtypes depending on its relation to the human papillomavirus (HPV): non-HPV-related, or differentiated PeIN, and HPV-related, or undifferentiated PeIN. The differentiated PeIN lesions are often associated with the inflammatory skin lesions known as lichen sclerosus or lichen planus.[2,3] Outside of their different pathologies, these two subtypes of PeIN can vary in appearance and may have different responses to treatment modalities.[4]

Due to the rarity of PeIN and the lack of standardized screening and diagnosis criteria, the incidence data for the disease is scarce. The most recent data depicts annual incidence rates of 0.5 per 100,000 men in England from 2007-2009, compared with 0.61 in the Netherlands in 2007, and 0.9 in Denmark from 2006-2008. Additionally, all three studies from which the data originated showed steadily increasing rates of incidence throughout their study periods.[5-7] Rippentrop et al. provides the only incidence data of PeIN on patients in the United States, and although they did not report an exact rate, they did find that from 1973 to 1998 the incidence of PeIN had increased whereas penile cancer rates decreased.[8] There is more concrete data for the incidence rates of penile cancer which can vary widely by country ranging from 0.0 to 7.26.[9] It is generally recognized that the rates of PeIN and penile cancer are conversely related depending on

the country or area. In less developed regions where penile cancer is more endemic, the rates of diagnosed PeIN are low, compared to more developed regions where PeIN predominates over penile cancer. The two likely explanations for this finding are differing circumcision rates and that patients will seek treatment sooner in well developed nations which can prevent progression of the disease.[10] Overall however, there is reason to believe that these incidence rates are underestimated due to the difficulty of recognizing the lesion by both the patient and clinician.[6,11] Although the incidence of PeIN in the United States is unknown, one could hypothesize that the incidence is approximately 1 per 100,000 or higher. The incidence of penile cancer in 2020 was 0.66 per 100,000 men, and with the increasing trend of PeIN incidence in the US in the Rippentrop et al. study and the inverse relationship of PeIN and penile cancer as discussed in the Canete-Portillo et al. study, there is some evidence to the claim.[12] However, further research is necessary to elucidate the true rates in the United States.

Regardless of PeIN subtype, these lesions are often indolent and superficial on the skin.[13] Rather than physical pain, penile lesions can elicit psychosexual damage and affect a man's sense of masculinity.[14] As a result, as many as 15-50% of men will delay care for up to a year after symptom presentation due to stigma and denial, which can be problematic for both treatment success and the progression of disease.[15] In a report of English patients from 1990-2009 there were no reported deaths directly associated with PeIN.[5] While there is no mortality associated with PeIN, progression to penile cancer could prove fatal as it has an estimated 5-year survival of 67%.[16]

As mentioned previously, the two mechanistic pathways for the development of PeIN are determined by the HPV status. In some studies, HPV DNA has been found in up

to 79.8% of PeIN lesions, with most cases involving highly oncogenic HPV strains such as HPV16 or HPV18.[17,18] In HPV-unrelated lesions, or differentiated PeIN, chronic inflammatory processes may promote carcinogenesis. Lichen sclerosus, a chronic inflammatory dermatosis, has been found to be associated with PeIN in 29-55% of cases depending on the country.[3] Other risk factors include prior disease on the prepuce, immunosuppressive drugs, prior penile surgeries, balanitis, genital warts, history of organ transplantation, and obesity.[3] PeIN is also more common in uncircumcised men but was not found to be a risk factor in a study by Daling et al.[19] HIV positive men report higher levels of PeIN than other men, often having HPV-related lesions, which is likely a result of their immunosuppression.[18,20,21] Prior research also shows a higher rate of undifferentiated PeIN among men who have female partners with cervical intraepithelial neoplasia (CIN), which is particularly true for the high-risk oncogenic HPV strains.[22,23] It is well known that an HPV infection is required for the development of CIN, and these findings suggest that there is likely a direct infection of male sexual partners resulting in the development of PeIN.[24]

There are several options for treating PeIN including topical medical therapy and various forms of surgery, but there is currently no gold standard treatment. Due to the rarity of PeIN, no randomized controlled trials (RCT) have been conducted, and the current basis for treatment has primarily come from literature reviews and case studies.[25] Excisional or surgical therapies offer a definitive treatment that fully removes the lesion; however, they often leave deformities and suffer from recurrence rates as high as 45.8% within the first year.[26] As a result, the first-line therapies are often topicals such as 5-fluorouracil (5-FU) and imiquimod creams because they are tissue-sparing options and

may have lower recurrence rates at 20% and 4% for 5-FU and imiquimod respectively.[27-30]

Due to the lack of robust evidence of efficacious therapies for PeIN, this study will focus on the treatment of PeIN using topical imiquimod. Imiquimod is an immunomodulator that can stimulate both the innate and cell-mediated immune pathways to provide strong antitumor and antiviral effects.[28] It is currently FDA approved for the treatment of genital warts, actinic keratosis, and superficial basal cell carcinoma.[31] It is currently used off-label for the treatment of PeIN, as well as other similar diseases such as cutaneous squamous cell carcinoma in situ, anal intraepithelial neoplasia (AIN), anogenital intraepithelial neoplasia (AGIN), and vulvar intraepithelial neoplasia (VIN).[29]

The literature that provides justification for the use of imiquimod for PeIN is scarce but promising. In 2017, Deen et al. published a literature review that compiled case studies and case series that utilized imiquimod as a monotherapy for biopsy proven PeIN with the goal of assessing the efficacy of the medication. Their results found that 63% of cases had a complete response, 8% had partial response, and 29% had no response. Moreover, imiquimod seemed to be more efficacious for BD and BP lesions with 88% and 75% complete response rates respectively, whereas EQ lesions only had complete response in 55% of cases. Due to the nature of the study, they could not control the imiquimod application regimens, so they divided their data into groups depending on the application frequency. They found that fewer applications over a longer treatment duration had higher rates of complete response: 81% in those that applied it less than 4 times per week compared to 68% for those who applied it at least 4 times per week. Although the results are promising, case reports are more likely to be published if there

are positive outcomes, so the true complete response rate of imiquimod may be overstated.[28]

Outside of PeIN, several studies have also utilized imiquimod for the treatment of VIN and AIN, both of which are analogous lesions to PeIN.[32,33] Mathiesen et al. conducted a double-blind, randomized placebo-controlled trial with imiquimod on VIN and found that 81% of patients had a complete response, 10% had a partial response, and 0 patients in the placebo group had any lesion reduction.[34] Kreuter et al. and Richel et al. both found that imiquimod had success in the treatment of AIN with an 81% complete response rate in a prospective cohort study and 24% complete response in an RCT.[35,36] Fox et al. conducted a double-blind, randomized placebo-controlled trial for the use of imiquimod on AIN lesions and had complete and partial responses of 14% and 28% in their patients respectively, compared to complete response in 4% of patients in the placebo group.[37] Lastly, a literature review from Mahto et al. investigated the use of imiquimod as a monotherapy for PeIN, VIN, and AIN and reported mean complete response rates of 70%, 51%, and 48% respectively.[29]

In addition to imiquimod, our study will utilize Mohs micrographic surgery (MMS) for all patients to ensure each patient receives equal care and eliminate the possibility of a false-negative result in a post-treatment biopsy. MMS is a staged process that involves complete microscopic evaluation of the excised specimens by the surgeon until there is no abnormal histology around the entirety of the lesion margins. This allows for a minimal amount of tissue loss, certainty that the primary lesion has been fully excised, and preferable cosmetic and functional results for the patient.[38] Currently, the use of MMS for PeIN demonstrates promising results with a cure rate of 94.7% from one

retrospective record review of two surgeons who treated 19 cases of PeIN.[39] In contrast to this, the cure rates of other surgical options are approximately 75%.[26] Although the evidence is minimal, MMS appears to be the superior treatment modality over other surgical options, but this procedure requires extensive training along with facilities properly equipped for MMS, so it is unsurprising that there is a lack of research of MMS for PeIN.

1.2 Statement of Problem

Penile intraepithelial neoplasia does not have a gold standard treatment due to the scarcity of research and a lack of randomized controlled trials. Consequently, current practice relies on case studies and literature reviews as a basis to guide therapy. Surgical options can fully remove a lesion in one visit, however the lesion can still recur and often leaves the patient with physical deformities.[25] For these reasons, tissue-sparing therapies such as topical medications are often considered first-line and also have data to suggest low recurrence rates.

As previously stated, a prior literature review on the utilization of topical imiquimod for treatment of PeIN has shown promise with complete response rates of 63% when used as a monotherapy.[28] However, the evaluated case studies in this review had varying treatment regimens and lacked additional characteristic information such as the HPV status, circumcision status, and the presence or history of inflammatory disease. Additionally, case studies are also more likely to be published if there are positive outcomes, so it is unclear if this complete response rate is an overestimate. There is a clear need for a randomized placebo-controlled trial to provide clarity on the true effectiveness of imiquimod for PeIN. Moreover, there is very little research on Mohs

micrographic surgery for PeIN despite data showing that it could be preferable to other surgical options.

1.3 Goals and Objectives

The primary aim of the proposed study is to provide robust data on the outcome of imiquimod treatment of PeIN and diminish the reliance on case studies to guide practice. A secondary aim is to provide additional data on the efficacy of MMS for PeIN, which should prove helpful in cases where topical therapies are not appropriate. A double-blind, randomized placebo-controlled trial would be novel for this disease and could provide the framework for similar future studies on other treatment options for PeIN. The primary outcome for the study is to assess the complete response rate of PeIN following a course of topical imiquimod 3 times weekly for 12 weeks compared to a placebo cream at the same frequency. All patients will be treated with MMS after completion of medical therapy. Complete response will be assessed both clinically and pathologically, with pathologic examination of tissue during MMS.

The secondary outcomes include partial response, complete clinical response with persistent histological disease, progressive disease, stable disease, incidence of recurrence after 12 months, time to recurrence over 12 months, time to clearance of lesion, occurrence of new secondary lesions unrelated to original lesion at first presentation, and incidence of side effects secondary to the interventions. This information could potentially demonstrate the effectiveness of MMS for PeIN, highlight the partial effectiveness of imiquimod, guide appropriate follow-up timelines, and provide data on the necessity of detailed inspections during regularly scheduled appointments over the treatment period.

1.4 Hypothesis

We hypothesize that men with PeIN who receive 5% imiquimod three times weekly for three months will have a significantly greater complete response rate one month following the treatment period than men who receive a placebo cream at the same frequency.

1.5 Definitions

Complete response is defined as complete clinical regression of the primary PeIN lesion and pathologically proven clearance of disease upon definitive surgical treatment with MMS.

Partial response is defined as at least a 30% decrease in diameter of the primary PeIN lesion from the baseline measurements after the 5% imiquimod or placebo treatments.

Complete clinical response with persistent histologic disease is defined as complete clinical resolution of the primary PeIN lesion from the baseline measurements but persistent histologic disease after the 5% imiquimod or placebo treatments.

Progressive disease is defined as at least a 20% increase in diameter of the primary PeIN lesion from the baseline measurements after the 5% imiquimod or placebo treatments.

Stable disease is defined as neither sufficient increase or decrease in the diameter of the primary PeIN lesion from baseline measurements to constitute as a partial response or progressive disease after the 5% imiquimod or placebo treatments.

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Chapter 2: Review of Literature

2.1 Introduction of Literature Search

The review of literature that pertains to PeIN, imiquimod, and MMS was conducted on several databases including Embase, Ovid (Medline), PubMed, and Cochrane Medical Library. Within each of these databases several search terms were utilized, and a thorough review of the relevant literature was performed. The following search terms were either used separately or in conjunction with one another: *penile intraepithelial neoplasia, Bowen's disease, Bowenoid papulosis, erythroplasia of Queyrat, PeIN, PIN, undifferentiated, differentiated, penile cancer, penile squamous cell carcinoma in situ, lichen sclerosus, lichen planus, treatment, therapy, imiquimod, topical, Mohs micrographic surgery, side effects, vulvar intraepithelial neoplasia,* and *anal intraepithelial neoplasia*. Only studies originally published in English were reviewed. There was no limitation on the year of publication in hopes to broaden our available knowledge on the subject.

2.2 Review of Empirical Studies Related to Imiquimod and Penile Intraepithelial Neoplasia

Review of Empirical Studies Solely Involving Imiquimod for PeIN

There are only two publications that studied the utilization of imiquimod as a monotherapy for PeIN. Both studies are literature reviews of case studies and case series, however there is little difference among them. The study by Deen was published in 2016 and reviewed all cases that Mahto et al. had previously evaluated in 2010. To be complete, we will evaluate both studies despite their similarities.

In 2010, Mahto et al. performed a literature review of all published cases where imiquimod was used as a monotherapy for PeIN, VIN, and AIN between 1997 to May 2009.[1] In this section, we will only discuss their findings regarding PeIN. They excluded all cases where imiquimod was used in combination with another therapy, as well as articles that involved children, genital warts, and non-English studies. Their primary outcomes were complete response, defined as complete regression of visible lesions with histological confirmation via biopsy, partial response, defined as >50% regression of the visible lesion, and no response, defined as <50% regression of the visible lesion.

Of the 17 articles they evaluated, 15 were case reports and 2 were cohort studies, for a total of 27 patients. The average age of the patients was 55 years (range 23-78 years), and among those men, four were HIV positive, and three of those men were on antiretroviral medications. The frequency in application varied among patients with the majority of patients either applying it on alternating days or applying it three times per week with minor variation. The treatment durations varied among the patients ranging from 3 weeks to 12 weeks, but the majority of patients used it for 12 weeks or 16 weeks. The follow-up durations ranged from 0 to 22 months (7.5 months average), and some cases did not report their follow-up data. They found that 21 (78%) patients had complete response, 5 (19%) had partial response, and 1 (4%) had no response. All four HIV positive men had a complete response. There were also no recurrences among the cases evaluated.

In 2017, Deen and Burdon-Jones performed a literature review similar to the Mahto et al. study, but focused entirely on articles where imiquimod was used as a monotherapy for PeIN.[2] They evaluated all cases previously mentioned in the Mahto et

al. study and added 12 more articles. They had the same exclusion criteria, inclusion criteria, and primary outcomes as the Mahto et al. study.

Of the 29 articles they evaluated, 22 were case studies and 7 were case series, for a total of 48 patients. They did not report any data on age or HIV status of the patients. They found that 30 (63%) of patients had complete response, 4 (8%) had partial response, and 14 (29%) had no response. There were 2 cases of recurrence after initial resolution of the lesion. In this study, they divided the patients based on application frequency into three groups: group 1 included those treated at least 4 times weekly (22 patients), group 2 included those who were treated less than 4 times weekly (16 patients), and group 3 included those where the treatment regimen was not reported (9 patients). Among group 1, the average total duration of treatment days per patient was 53, with 0.53 average applications per day. Among group 2, the average total duration of treatment days per patient was 113, with 0.41 average applications per day. Group 3 did not have this data. In group 1, 15 (68%) patients had complete response, 1 (5%) patient had partial response, and 6 (27%) had no response. In group 2, 13 (81%) patients had complete response, 2 (12.5%) patients had partial response, and 1 (6%) patient had no response. In addition to this, they also evaluated the efficacy among different types of PeIN. For EQ, 17 (53%) patients had complete response, 3 (9%) patients had partial response, 12 (38%) patients had no response, and there were 6 incidences of recurrence. For BD, 7 (88%) patients had complete response, 0 patients had partial response, 1 (12%) patient had no response, and there were no recurrences. In BP, 6 (75%) patients had complete response, 1 (12.5%) patient had partial response, 1 (12.5%) patient had no response, and no there were no recurrences. The follow-up durations ranged from 0 to 36 months, and some cases did not

report their follow-up data. In comparison to the Mahto et al. study, the addition of analyzing these subgroups by frequency of application provides important information on to best utilized imiquimod for PeIN.

There are a few details to highlight from these two studies despite the limitations of a literature review. It is evident that application frequency and duration have some effect on the percentage of patients who achieve complete response. In the Deen study, group 1 had a higher application frequency with a shorter duration of therapy and found that only 68% of patients had complete response with 27% of patients having no response. Contrasting this to group 2 who had lower application frequency and longer treatment duration, 81% of patients had complete response and 6% of patients had no response.[2] Based on these findings, it seems that applying imiquimod less than 4 times per week for approximately 3 to 4 months could be the ideal treatment regimen. Additionally, it seems that EQ is slightly more resistant to imiquimod and has a higher chance of recurrence compared to BD and BP. The Mahto et al. study suggested that HIV positive men respond just as well to imiquimod compared to any other patient.[1] Notably, neither study reported any data on other important risk factors including, HPV status, HPV DNA, or circumcision status. Discerning risk factors could prove vital when choosing the best treatment option for a patient. The evident limitation of these interpretations is that they are based on small sample sizes, however it is the only data available to interpret at this time. Both literature reviews further potentiate the necessity of an RCT to standardize the treatment regimen and follow-up periods.

Brief Review of Empirical Studies Involving a Combination of Imiquimod and Another Therapy for PeIN

Several studies have investigated the use of imiquimod in combination with other viable treatments for PeIN. The review on these studies will be succinct and will serve as tool to demonstrate that imiquimod can serve as a possible adjunctive therapy or second line treatment. Additionally, these are some of the only other available literature on imiquimod for PeIN, so it is important to highlight the findings in these studies to provide a more holistic view on the topic of interest.

In 2012, Alnajjar et al. conducted a 10-year retrospective review of all patients who presented with PeIN at their UK-based medical center.[3] Their focus were those who received topical medications for treatment, of which they found 42 patients who received 5-FU as a first line therapy, and 9 patients who received imiquimod. In this study, imiquimod was either utilized when previous 5-FU treatment had failed or used as a second-line therapy after partial success of 5-FU. Their outcomes were complete response, defined as resolution of the lesion, partial response, defined as reduction in size or visibility of lesion, and no response, defined as no improvement in lesion size or visibility. Their results showed that among those who only received 5-FU, 21 (50%) patients had complete response, 13 (31%) patients had partial response, and 13 (31%) patients had no response. Among those who received imiquimod, 4 (44%) had complete response, 0 had partial response, and 5 (56%) had no response. Of the 4 patients who had complete response with imiquimod, two had prior failure of 5-FU, and two used imiquimod as a second line therapy. It is unclear why these patients benefited from

imiquimod despite resistance to 5-FU, but it is possible that it could be attributable to certain risk factors or that the patients were poorly adherent to the treatment regimen.

In 2017, Shaw et al. conducted a retrospective study of the patients diagnosed with PeIN who received cryotherapy as a treatment modality at the Dermatology service of Memorial Sloan Kettering Cancer Center.[4] This study identified 8 patients, all of whom received cryotherapy and imiquimod. The cryotherapy sessions averaged 5.1 sessions (range 2-11). The imiquimod regimen was 3 to 5 times per week for 8 weeks, then a maintenance phase of 1 to 3 times per week to prevent recurrence, which resulted in an average treatment duration of 7.8 months (range 2-13 months). This study also collected several baseline factors including PeIN type, HIV status, HPV status and DNA, and circumcision status. All patients achieved complete response after the conclusion of the therapy and none had recurrence of the disease, but the small sample of patients was far from homogenous. 4 patients received 5-FU in addition to the cryotherapy and imiquimod. 3 patients received some form of excision which included circumcision, Mohs surgery, or laser excision. Of the 5 patients who were tested for HPV DNA, all five had HPV 16/18 DNA present, which is notably the most oncogenic strains. 3 patients were HIV positive and on antiretroviral medication, but all 3 used 5-FU and 1 patient required circumcision. Although this study was thorough in their collection of descriptive data and treatment regimens, it is difficult to pinpoint which of these therapies was providing the most benefit.

In 2017, Torelli et al. performed a case series of 10 patients with erythroplasia of Queyrat who were initially treated with imiquimod then followed by carbon dioxide laser ablation.[5] Their exclusion criteria were lesions that had recurred within the previous year,

lesions on the penile shaft, urethral extension beyond the meatus, and presence of other malignancies. This study assessed the complete response rate with a pre and post-treatment biopsy. The treatment regimen was the application of imiquimod on alternating days for 12 weeks. HPV and HIV statuses were assessed for all patients, of which 7 had HPV and one was also HIV positive. The results of the study found that 6 patients had complete response, 2 had no response, and 3 had progressive disease. Notably, 6 of the 7 patients who had HPV had complete response. None of the patients who achieved complete response had evidence of recurrence during their follow-up period which averaged 26 months (range 12-58 months). Without a comparison group, it is unclear if the complete response is due to the imiquimod, laser, or both. Past studies have shown that carbon dioxide laser on its own can also achieve complete responses in PeIN.[6] The authors explained that they chose to only include EQ lesions due to their higher chance of progression to invasive disease, but the exclusion of other PeIN types such as BD and BP limits the generalizability of this study. This study does provide more support that imiquimod may have more efficacy against HPV-related PeIN lesions, but the addition of the laser therapy obscures this interpretation.

Overall, it is difficult to conclude that imiquimod is a suitable monotherapy based on these three studies, but it is evident that imiquimod can either provide some additional therapeutic response or is at least not detrimental to the care of these patients.

Brief Review of Empirical Studies Involving Imiquimod for Analogous Lesions to PeIN

Although the data for topical imiquimod use on PeIN is scarce, there are more robust studies on its use for other similar HPV-related diseases such as anal

intraepithelial neoplasia and vulvar intraepithelial neoplasia, both of which share several characteristics with PeIN and have similar precancerous changes.[7,8] This section will discuss the other findings in the Mahto et al. study along with two Cochrane reviews, one of which focused on AIN and the other of which focused on VIN.

As previously mentioned, Mahto et al. conducted a literature review on the use of imiquimod as a monotherapy for PeIN, AIN, and VIN.[1] The review of AIN studies discovered 5 articles, of which 3 were case reports, 1 was a prospective non-randomized study, and one was an observational cohort study, for a total of 32 patients. The treatment regimens for all patients was 3 times per week and reduced as needed for side effects. Notably 29 of the patients were HIV positive and many of those men were on antiviral medication. The results showed that the mean complete response rate was 48%, partial response rate was 34%, and 36% of the patients had recurrence. The review of the VIN studies discovered 20 articles, of which included 2 RCTs, 8 uncontrolled case series, 9 case reports, and 1 review article. The controlled and uncontrolled trials included 202 patients. The application frequencies for these patients ranged from 1 to 3 times per week. The results showed that the mean complete response rate was 51%, partial response rate was 25%, and 16% of patients had recurrence. This data is promising for the utilization of imiquimod as a monotherapy for AIN and VIN as the source of the data comes from more robust study designs.

In 2012, a Cochrane review by Macaya et al. investigated interventions for anal canal intraepithelial neoplasia (AIN). Their inclusion criteria were RCTs investigating any type of intervention, however the only study that fit the criteria was by Fox et al. who studied the effect of imiquimod versus placebo on AIN.[9] This study included 53 patients,

all of whom were HIV positive and men who have sex with men (MSM). The application frequency was 3 times weekly for 4 months. The results of the study found that 4 of 28 patients in the imiquimod group had complete response compared to 1 of 25 in the placebo group, and these results were not statistically different (RR 3.57, 95% CI 0.43 to 29.87). Additionally, 8 patients in the imiquimod group showed downgrading in their lesion to low-grade intraepithelial neoplasia compared to 0 in the placebo group, but these results were also not statistically significant. (RR 15.24, 95% CI 0.92 to 251.29). The Cochrane review considered the risk of bias for this study to be moderate. The randomization sequence and allocation of treatment was determined to be unclear due to the vague description of both the sequence generation and randomization process. They stated that an intention to treat analysis was performed but failed to describe the allocation of the patients lost to follow-up. There was possible selective reporting because there is no access to the protocol of the study. Lastly, the baseline characteristics were different among the two treatment groups.[9] Overall, this study provides only suggestive evidence for imiquimod on AIN and it is evident that there may be some bias in this study. In addition to this, all patients were HIV positive on antiretroviral medication, so these patients may require a more intense treatment regimen or a combination of therapies to fully eradicate their disease.

In 2016, a Cochrane review by Lawrie et al. investigated interventions for vulvar intraepithelial neoplasia.[10] Their inclusion criteria were either RCTs or non-RCTs that had concurrent comparison groups and a multivariate analysis of the baseline characteristics. Among the trials they reviewed, there were three RCTs that compared imiquimod to placebo by Sterling et al, Mathiesen et al, and van Seters et al.[11-13] There

were a total of 104 patients among these three studies. The results found that 36 of 62 patients had complete response in the imiquimod group compared to 0 of 42 patients in the placebo group (RR 14.40, 95% CI 2.97 to 69.80). They concluded that patients allocated imiquimod were more likely to achieve complete or partial response compared to the placebo group (RR 11.95, 95% CI 3.21 to 44.51). The only study to have significant follow-up was the van Seters et al. trial, and they found that only 1 of the 9 patients who achieved complete response had recurrence after four years. In addition, the van Seters et al. study found that 15 patients in the imiquimod group had cleared their HPV after the treatment period compared to 2 in the placebo group (RR 0.43, 95% 0.26 to 0.72).[13] The Cochrane review concluded that the Mathiesen et al. and van Seters et al. studies had low risk of bias, and the Sterling et al. study had a high risk of bias.[10] The Mathiesen and van Seters studies were both double-blinded and were fully transparent on their methodology, whereas the Sterling study was only published in the abstract form. The issues with these studies are that none of them included immunocompromised patients, so it is unclear how they would respond to these treatments. Of the three, only one study followed the patients for 5 years, so the recurrence data is likely inadequate to make any solid interpretations. Overall, this data does show promise for imiquimod as a monotherapy for VIN and it is supported by strong evidence through 3 separate RCTs.

2.4 Review of Side effects of Imiquimod

The side effects of imiquimod are better understood on extragenital sites, but from the small amount data available imiquimod seems to be generally well-tolerated for PeIN.[14] The typical side effects of imiquimod are burning, erythema, irritation, itching, tenderness, bleeding, crusting, and hypopigmentation at the application site.[15] In addition

to this, some patients may experience flu like symptoms, headache, and myalgia.[14] In the Shaw et al. study of 8 patients who received imiquimod, they reported that patients experienced mild redness and irritation at the site of application with no severe skin reactions.[4] However, they did not specify how many patients experienced side effects, and all of these patients were being concurrently treated with cryotherapy, 5-FU, or both. In the Torelli et al. study they treated 10 patients with imiquimod for PeIN, and all of their patients experienced burning erythema at the site of application, but none of them reported fever, myalgia, or leukopenia.[5] One patient had to stop treatment due to scrotal ulceration.

Mild local toxicity seems to be common when imiquimod is used for PeIN, however these patients can usually continue their treatment course. In the event that side effects do occur, patients are often prescribed topical steroids or advised to use cold compresses so they can continue therapy.[2] Overall, the data suggests this is a safe option for PeIN and there are methods to alleviate the effects when they arise.

2.5 Review of Empirical Studies Involving Mohs Micrographic Surgery for Penile Intraepithelial Neoplasia

Mohs micrographic surgery can be a less invasive procedure compared to wide local excision, and it can be more precise with the margins of the lesion to preserve healthy tissue. To date, there are only two retrospective case series investigating MMS for PeIN, but the data from these two studies does show promise.

In 1988, Brown et al. reviewed all the cases from their clinic in which Mohs surgery was performed on genital tumors.[16] In their search, they found 24 cases, and 4 of which were Bowen's disease on the penis. Their primary outcome was to investigate the

recurrence rates among these genital lesions. The results found that none of the 4 patients with penile BD had recurrence after an average follow-up of 2 years. However, they did not report the exact follow-up time for each individual, and they also mentioned that 4 of the 24 patients were lost to follow-up but did not specify which patients. This study has clear evidence for bias with the lack of details surrounding the follow-up time and patient characteristics, but it does indicate that MMS could be efficacious for PeIN based on the results.

In 2016, Machan et al. published a more robust retrospective record review that investigated patients who received MMS for their penile tumors.[17] Among their sample, 23 of the patients had PeIN with follow-up data on 19 of those patients. The primary outcome of this study was also recurrence rates following MMS. Only 1 of the 19 patients had recurrence after a mean follow-up time of 97.4 months, indicating a cure rate of 94.7%. The one case of recurrence occurred after 9 months and was successfully treated with a second MMS. This study provided a much larger sample size and longer follow-up times and is most likely the current gold standard research regarding MMS for PeIN.

In conclusion, the data presented on MMS for PeIN is promising albeit scarce. Neither study reported any baseline characteristics such as HPV status, HIV status, or circumcision status, so it is unclear if this procedure is more efficacious for patients with certain risk factors or specific PeIN types. However, the follow-up period for these cases is generally quite long and seems like enough time to follow the course of this disease. By combining the data of these two studies, the recurrence rate following MMS is 4.3% with an average follow-up time of approximately 60.7 months.[16,17] Despite the small

sample size, this figure does warrant further research into this subject to better elucidate the true efficacy and recurrence data.

2.6 Review of Complications of Mohs Micrographic Surgery

The literature on MMS for penile lesions is scarce, but there is an abundance of research dedicated to its utilization on other anatomical areas along with the complications that occur. This in-office procedure is considerably safe with a complication rate of approximately 1.64%. Of these complications, most of them are either hematoma formation or postoperative hemorrhage and are easily managed with compression, observation, or occasionally drainage. Rarely, graft or flap necrosis can occur, but these were managed with wound care and observation with good success.[18] The incidence of major complications is also extremely rare, and in one study of 3937 patients, only one developed a gastrointestinal hemorrhage secondary to naproxen use that was prescribed postoperatively.[19] Postoperative pain is usually tolerable and successfully managed with acetaminophen, ibuprofen, opioids, or a combination of these medications.[20]

Regarding MMS for PeIN, the previously discussed Brown et al. and Machan et al. studies provide the only data on the subject. In the Brown study they reported that none of the patients had significant postoperative complications and that most of the defects were healed by secondary intention. No other details were stated in the article.[16] In the Machan study, 4 of the patients with PeIN had urethral involvement, all of which developed urethral stricture and required urethral dilation postoperatively. One patient required urethral reconstruction. However, all 4 cases eventually had urethral function restored.[17]

MMS is a viable option for therapy of these lesions, but the anatomical considerations of operating on penile tissue can pose some difficulty. The penile anatomy is contoured and the skin much more elastic than most. Additionally, the anatomy can be distorted with anesthesia and edema during the procedure making it more challenging to discern the true margins of the tumor.[17] Despite these difficulties, this should not dissuade a patient from undergoing this procedure. With enough expertise, training, and clinical judgement, it appears that MMS can be a viable option for penile lesions.

2.7 Review of Methodology of Imiquimod and Mohs for Penile Intraepithelial Neoplasia

Study Design and Confounders

Although several studies have tried to determine the efficacy of imiquimod for PeIN through literature reviews and retrospective record reviews, the ideal design would be a double-blind, randomized placebo-controlled trial.[1,2] These trials are the gold standard for determining the efficacy of a medication, as the results are more generalizable and diminish both information bias through blinding and selection bias with the randomization process. Unfortunately, there are no RCTs to reference when designing a study around imiquimod and PeIN, but there are several robust RCTs of which studied imiquimod as a monotherapy on the analogous lesions of AIN and VIN.[9,12,13]

Another benefit to RCTs is that confounders can be controlled by collecting certain baseline characteristics and conducting specific analyses. In prior literature reviews on PeIN, they did not include many patient characteristics because the original cases did not report this information.[1,2] By recruiting patients for a study, it is possible to collect any baseline data desired. In past studies regarding imiquimod for AIN and VIN,

they collected information on important risk factors such as HIV status, HPV status, HPV DNA, and smoking status. These studies were then able to perform a multivariate analysis to control for confounders in their final results and strengthen their conclusions.[9,12,13] For a study involving imiquimod for PeIN, these same risk factors should be collected with the addition of PeIN type and circumcision status. Certain PeIN types have been shown to be more susceptible to imiquimod, and circumcision status is a known potential risk factor.[21,22] As seen in the Deen et al. study, EQ does not respond as well as BD or BP to imiquimod, so stratifying the randomization process so that there are equal number of patients with EQ in each group would be imperative to reduce a potential confounder.[2] The difference in response to imiquimod in BD and BP is much less apparent, so it would not be necessary to ensure equal groups of these lesions.

Interventions

Virtually all known studies regarding imiquimod as treatment for PeIN use the 5% dosage.[1-5] Additionally, many studies investigating imiquimod for VIN and AIN also use the 5% dosage.[1,9,11-13,23] However, the application frequency and treatment duration is variable across all of these studies.

For AIN and VIN studies, the variation among treatment regimens was only slightly different. An RCT by Fox et al. investigating imiquimod for AIN had an application frequency of 3 times per week for 16 weeks. The results showed a complete response rate of 14%.[9] An RCT by van Seters et al. investigating imiquimod for VIN had an application frequency 2 times per week for 16 weeks. The complete response rate in this study was 35%.[13] Lastly, another RCT by Mathiesen et al. investigating imiquimod for VIN had varying application frequencies depending on how the patients tolerated the

medication. They started at once a week for 2 weeks, then increased to 2 times a week for 2 weeks, then 3 times a week for 12 weeks, for a total of 16 weeks in duration. The results of this study found a complete response rate of 81%.[12]

For the PeIN studies, both the application frequency and duration of treatment varies. In the Shaw et al. study which included 8 patients, the application frequency ranged from 1 to 5 times per week and the duration of treatment ranged from 2 to 13 months. All patients in this study achieved complete response, however this cannot be solely attributed to the imiquimod as some of the patients were on multiple therapies including cryotherapy and 5-FU.[4] In the Torelli et al. study which included 10 patients, the application frequency was alternating days for 3 months. The results found that 60% of patients had complete response, but this study also had the addition of laser therapy before final biopsy.[5] As previously stated, the results of the literature review by Deen indicated that the patients who applied imiquimod less than 4 times weekly had a complete response rate of 81% and average of 113 days compared to 68% in the group who applied at least 4 times weekly for an average of 53 days.[2] The two key features of this study is that it only included cases in which imiquimod was a monotherapy, and it has the largest sample size to date (n=48). For these reasons, this is the best study available to determine the treatment regimen that is most likely to achieve complete response in future research. These findings are corroborated when considering the aforementioned studies involving AIN and VIN in which treatment also did not exceed 3 applications a week and saw varying levels of success in complete response.[9,12,13]

Post-Treatment Options

In the three RCTs involving imiquimod for AIN or VIN, they all provided different treatment options to the non-responders and placebo group. In the Fox et al. study, the partial responders, non-responders, and placebo group were offered a 4 month course of imiquimod at conclusion of the study.[9] In Mathiesen et al. if anyone in the treatment group or placebo group patients had persistence of VIN2 or VIN3 after post-treatment biopsy, they were offered imiquimod, laser ablation, or surgery.[12] In the van Seters et al. study, the patients continued to be randomized until completion of the 12 month follow-up. They did not report what was offered to non-responders or the placebo group, however they mention that all patients were made aware at the beginning of the study that surgery is the treatment of choice for VIN.[13]

For an RCT involving imiquimod and PeIN, the best treatment to offer any non-responders or placebo group patients would also be surgery, with preference for MMS. MMS has a recurrence rate of approximately 4.3% after 5 years compared to 19.0% after 5 years in other surgical options including wide local excision, laser excision, circumcision, and glansectomy.[16,17,24] MMS would be the safest with regards to recurrence rates and is the best tissue-sparing option.

Study Population and Selection Criteria

Since there are no RCTs on imiquimod for PeIN, there are no direct studies to reference when considering inclusion and exclusion criteria. The selection criteria in the preexisting literature reviews and case series are kept to a minimum as they typically only required a biopsy-proven diagnosis of PeIN.[1,2] One case series had more detailed exclusion criteria that excluded patients with recurrent lesions from within one year prior, urethral extension beyond the meatus, Bowen's disease, and previous or concurrent

malignancies.[5] These studies do not serve much purpose in guiding the selection criteria for a future study on imiquimod for PeIN, so the next best available reference material would be reviewing RCTs on imiquimod for AIN and VIN.

In an RCT investigating imiquimod for AIN in HIV positive men, they focused their exclusion criteria primarily on CD4 nadirs, length of antiviral medication usage, and lack of prior imiquimod use in the anal canal.[9] In the RCT by Mathiesen et al. on imiquimod for VIN, they included all patients with biopsy confirmed VIN2 or VIN3. They excluded any patients with evidence of invasive disease, HIV positive patients, use of immunosuppressive medications, and a positive pregnancy test.[12] Similarly, in the RCT by van Seters et al. on imiquimod for VIN, they included patients 18 years or older with biopsy-proven VIN2 or VIN3, but also stipulated that the patients must use contraceptives and be premenopausal to avoid potential teratogenicity risk. They excluded any patients with evidence of invasion, history of cancer or inflammatory disease of the vulva, pregnancy, immunodeficiency, and previous treatment for VIN or genital warts in the prior month, sensitivity to imiquimod, and inability to speak Dutch or English.[13] Using these three studies, it is possible to create a suitable selection criteria for a similar study done focused on PeIN.

The one inclusion criteria that is ubiquitous among all studies mentioned, and an obvious requirement, would be the necessity of biopsy-proven diagnosis of the lesion of interest. PeIN is difficult to diagnose based on presentation, even when using dermoscopy, so a biopsy is often necessary.[25] The age for inclusion should be 18 years or older since there have been no documented cases of PeIN in adolescents yet, and it is unclear how they would respond to treatment. Regarding exclusion criteria, excluding

those with evidence for invasion would be beneficial as they may have a more serious tumor and require more aggressive treatment. The one topic where there is a clear difference in inclusion and exclusion criteria is whether to include HIV positive patients and those on immunosuppressive medications. Prior data indicates that HIV positive men can still achieve complete response with imiquimod as a monotherapy, so it seems that not all patients would require more aggressive therapy.[1] Additionally, HIV positive men are disproportionately affected by PeIN compared to immunocompetent men.[26-29] Based on these findings, it seems that the addition of HIV positive men in PeIN studies would be greatly beneficial to that population and excluding them would significantly decrease the patients eligible for recruitment. Since there is no data to support that HIV status results in a significant difference in clinical response to imiquimod, there would be no need to stratify these patients into equal numbers between groups for a study.

Recruitment and Sampling

The biggest challenge to conducting any study involving PeIN is the recruitment phase. The incidence of this disease is low and varies depending on the country of interest, so the only possible way recruit enough patients for an RCT would be to conduct a multicenter study.[30-34] The two previously mentioned RCTs investigating imiquimod for VIN required 3 years at one center and 2 years at two centers, and VIN has an incidence of approximately 5.0 per 100,000.[12,13,35] The clear solution to recruiting enough patients would be to conduct a multicenter study. Despite the logistical challenges, conducting a multicenter RCT does provide some benefits to the validity of the study. The obvious benefit is a larger sample size at a quicker recruitment rate that would not be achievable at one or two locations. A multicenter RCT is also considered more

generalizable due to a larger, more heterogeneous sample population.[36] Performing a multicenter RCT for PeIN would be novel, and likely the only method of gathering any meaningful data in a reasonable amount of time.

Because recruiting patients for PeIN would already prove difficult, there would be no reason to randomly sample these already rare patients. The best method to sample PeIN patients would be consecutive sampling. This would ensure a large enough sample size that provides sufficient power to detect the expected effect size. All three previously mentioned RCTs involving imiquimod for AIN and VIN utilized this sampling technique.[9,12,13] The disadvantage to this sampling technique is that it would negatively impact the validity of the research due to lack of randomization and it may not be representative of the population. However, this issue would be alleviated by a multicenter study as previously suggested.

Primary and Secondary Outcomes

All studies involving imiquimod for PeIN use complete response as a primary outcome.[1,2,4,5] These studies specify that complete response is defined by complete histological absence of disease confirmed by biopsy after the treatment period. This outcome is likely chosen over others, such as partial response, because demonstrating that imiquimod can fully eradicate PeIN without the assistance of other therapies proves its capabilities as a monotherapy. Complete response both clinically and histologically is also the gold standard for cancer therapy in general. Although the primary outcome is the same across many studies, the choice of secondary outcomes often varies.

Another commonality among many of these studies is defining the secondary outcome of partial response to treatment, but these definitions are not homogenous. In the Deen and Mahto et al. literature reviews of imiquimod for PeIN, they defined partial response as 50% regression or more of the visible lesion.[1,2] In both the Shaw et al. case series of imiquimod and cryotherapy for PeIN and the Torelli et al. case series of imiquimod and carbon dioxide laser for PeIN, neither study included partial response as a secondary outcome.[4,5] Notably, neither study reported any instance of a partial response, but it is unclear if they decided not to include those patients in the results, or if they truly did not experience that outcome. In the van Seters et al. and Mathiesen et al. RCTs of imiquimod for VIN, they both defined partial response as histological regression of the lesion to a lower grade of VIN.[12,13]

For a future study involving PeIN, defining partial response as reduction in diameter by 30% or greater would be acceptable and meaningful. Size regression of that extent is both clinically relevant and could provide evidence for the possibility of using imiquimod in combination with other treatments. The basis for this definition is from the RECIST criteria which is set of guidelines created in an effort to standardize the measurements of changes in tumor burdens for the clinical evaluation of treatments.[37] Using this standardized criteria eliminates any guessing work for what constitutes partial response, and would eliminate any potential bias when researchers try to create their own definition of partial response that may produce clinically insignificant results.

Many studies attempt to investigate the recurrence rates after application of imiquimod, but ultimately the time, resources, and patient adherence dictates the follow-up times necessary to study this outcome. The Mahto et al. study had a mean follow-up of

7.5 (range 1 to 22 months) while the Deen et al. study had a mean follow-up of approximately 12.3 months (range 1 to 48 months).[1,2] The van Seters et al. study had a follow-up time of 12 months.[13] Ideally, to obtain the most meaningful recurrence rates, the follow-up times should be near 2 to 5 years, but the rarity of the disease and resources available may limit the feasibility of such a study. Instead, a follow-up time of 12 months would likely be sufficient for an initial study on efficacy of imiquimod, leaving the potential for a future study that investigates recurrence rates over several years.

Lastly, another meaningful secondary outcome for a study on PeIN would be incidence of side effects secondary to imiquimod. Both the Shaw et al. and Torelli et al. studies reported that their patients experience side effects to the imiquimod.[4,5] The van Seters et al. study recorded incidence of side effects and found that patients receiving imiquimod had a statistically significant higher incidence of side effects compared to placebo for vulvar pain or pruritis ($p < 0.001$), mild-to-moderate erythema ($p < 0.001$), and edema ($p < 0.001$).[13] Knowing how many patients may experience side effects allows for better patient education if a clinician was to prescribe this medication.

Adherence

The adherence to imiquimod is vital to obtain clinically relevant data and draw conclusions. The Shaw et al. case series on PeIN and the two RCTs on VIN utilized monthly visits to assess adherence, side effects, and clinical progression.[4,12,13] Both the Shaw and Mathiesen studies did not report any non-adherence among their patients, and the van Seters study only had two patients discontinue treatment. Contrasting this to the Fox et al. RCT on AIN, they only recommended the patients make a diary for side effects and did not report if patients made any visits during the treatment periods.[9] The Fox study

ultimately had 11 (17%) patients drop out, and the most common reason reported was unhappiness with the possibility of receiving placebo. By comparing these studies, it seems that the best method to better ensure adherence among the patients for a future PeIN study would be a mixture of an at-home diary to self-report side effects and monthly visits with their clinicians. The diary would fill the gaps between the monthly visits and the meetings with clinicians would provide positive reinforcement for continuing therapy.

Sample Size

The sample sizes for an RCT on imiquimod for PeIN would entirely depend on the expected effect size, but if the effect is large enough, a small sample size could be sufficient. The Fox et al. RCT for AIN had a sample size of 64, the van Seters et al. RCT for VIN had a sample size of 52, and the Mathiesen RCT for VIN had a sample size of 32.[9,12,13] Despite these small sample sizes, all three studies managed to produce statistically significant data for their primary outcomes. The expected effect size of imiquimod for PeIN would be 63% based on the Deen study. It would be beneficial to base the expected effect size of the placebo from a study by Swetter et al. They reported that 24% of their patients had complete response only after receiving biopsies for their squamous cell or basal cell carcinomas without additional treatment.[38] Although these lesions are not completely analogous to PeIN, this provides a placebo effect size likely higher than the true expected value which would strengthen the results and provide a margin of error if the effect size of imiquimod was overestimated. Using these two studies, this would require a minimum of 50 patients with 25 patients in each arm with an

alpha of 0.05 and power of 80%. Given the right study design, this sample size is a feasible amount to recruit.

2.8 Conclusion

In conclusion, imiquimod has shown promise for its efficacy in treating PeIN as well as other analogous lesions including AIN and VIN. In addition to imiquimod, Mohs micrographic surgery has some data demonstrating that it can successfully remove the lesion and provide a low recurrence rate compared to other surgical options.[16,17,24] Implementing imiquimod as a standard therapy for PeIN is likely hindered by the lack of RCTs, however by comparing past literature reviews on imiquimod, and other RCTs which utilized imiquimod on analogous lesions, a well-designed and feasible trial could be accomplished.

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Chapter 3: Study Methods

3.1 Study Design

We will conduct a multicenter, double-blind, randomized placebo-controlled trial for men who present with primary, biopsy-proven PeIN lesions. These lesions will not have been previously treated or recurrent lesions. This study will compare the efficacy of 5% imiquimod cream to a placebo cream and assess for complete clinical and histologic response after the treatment period. Following the treatment period, all patients regardless of clinical outcome will undergo Mohs micrographic surgery, which will serve as a method to assess for any remaining histological presence of disease and remove any part of the lesion that remains after treatment.

Patients who present to their clinicians with lesions suspicious for PeIN will undergo a biopsy, and once confirmed, they will be asked to participate in the study and start the treatment period if they agree. The treatment period will last 3 months, after which there will be a 1 month waiting period for any side effects to resolve. After these 4 months, all patients will undergo MMS. The study will have a follow-up period of 12 months during which patients will meet with their clinicians every 3 months to assess for signs of recurrence.

3.2 Study Population and Sampling

Our study population will consist of men ages 18-80 who present with *de novo,* biopsy proven PeIN lesions of any classification. These men will be recruited from the following hospitals and institutions in the New England area where MMS is available: Yale New Haven Hospital, UConn Health, Hartford Hospital, Smilow Cancer Hospital, Massachusetts General Hospital, Rhode Island Hospital, Dartmouth-Hitchcock Medical

Center, Brigham and Women's Hospital, Tufts Medical Center, UMass Memorial Medical Center, and MaineGeneral Medical Center. At each of these clinical sites, we will recruit patients from both the dermatology and urology clinics, as both fields of medicine care for and manage PeIN. We will utilize a consecutive sampling technique to ensure we recruit the target of 56 individuals within the 8-month time span.

3.3 Inclusion Criteria

Inclusion for this study consists of the following criteria. The patients must be men between the ages 18-80 who present with primary, biopsy proven PeIN lesions of any size or classification. The patients must have a method of communication so that the researchers can contact them periodically throughout the study. The patients must be able to apply the topical creams themselves or have a caretaker apply the cream for them as directed. The patients must speak English so that they understand the consent form and so that someone is able to communicate with them during the treatment period.

3.4 Exclusion Criteria

Exclusion for this study consists of the following criteria: men ages 18-80 who present with a recurrent PeIN lesion or a PeIN lesion that has already been treated with any therapy, a patient who has used imiquimod for PeIN or genital warts in the last year, biopsies that have evidence of invasive carcinoma, patients with an allergy or contraindication to imiquimod therapy, patients that are not suitable candidates for MMS based on the clinical judgement of their providers, and patients with an uncontrolled inflammatory disease of the genitalia.

3.5 Subject Protection and Confidentiality

This study will be reviewed by the Institutional Review Board (IRB) at Yale University per the IRB policy 100, along with the IRB at each respective medical center and hospital included in this study. Per IRB policy, informed consent will be required from all patients prior to the induction into the study. The patients will be informed on the goal of the study, potential risks, potential benefits to them and the scientific community, the time course of the study, and the alternative treatment options. All patients will be ensured that their medical records and information will be kept confidential and within password-protected, encrypted servers. These servers will only be accessed by research personnel and for the intention of this study alone. This study will be in concordance with Health Insurance Portability and Accountability Act (HIPAA) regulations, and patients will be informed of their HIPAA rights before study initiation. All clinicians and research personnel involved in the study will undergo HIPAA training if they are not already HIPAA compliant. The study proposal submission will include all pertinent information regarding funding, the hiring of research personnel, training for research personnel, and methods of data acquisition and retention. A sample consent form is located in Appendix A.

Patients will be informed that they are eligible to drop out of the study at any point during the treatment period or follow-up period. They will be informed that dropping out of the study will not interfere with their established care, and that they may continue the current care with their respective clinicians if they desire. Similarly, any research personnel involved in the study may drop out if they desire. Information on payment forfeit for these individuals will be detailed in the IRB form.

3.6 Recruitment

We will conduct an 8-month recruitment period at all aforementioned clinical sites. This will allow for a full 3-month treatment period, 1-month for side effect resolution, and 1 full year of follow-up within the 2-year timeline of the proposed study. The patients will be primarily recruited through the clinicians taking part in this study and referrals from physician colleagues at their respective institutions and communities. Therefore, we will focus our advertisement efforts towards the clinicians at these institutions. Patients will be asked to enroll in the study once the clinicians have confirmed their diagnosis of PeIN through a biopsy. At that time, the patients will be provided a consent form and informed in detail regarding what the study entails and its risks and benefits. The patients will be informed that there is a small stipend for participating in the trial, and all medication, surgical costs, and miscellaneous travel costs will be covered.

3.7 Study Variables and Measures

The intervention will be 5% imiquimod cream in its generic form. The patients will be advised to apply the imiquimod cream 3 times weekly for 3 months. The cream will be kept on for 12 hours when applied and washed off thoroughly afterwards. The placebo topical cream will also be applied at the same frequency. The placebo cream will be manufactured and produced by the same company that produces the topical imiquimod. After three months, the patients will stop using the creams, and will wait one month for any side effects to resolve before they all undergo MMS.

The primary outcome will be complete response, characterized by complete clinical resolution of all visible lesions and histologic resolution confirmed through

MMS. MMS will be used in place of post-treatment biopsies as scouting biopsies for residual histologic disease in the absence of visible clinical lesions has poor sensitivity.[1] In addition, providing MMS to all study participants will ensure that all patients receive the standard of care in complete surgical lesion removal. Because MMS is an inherently tissue-sparing procedure, patients with complete response to medical treatment will undergo a minimally invasive procedure. At the conclusion of MMS, all specimens will be submitted to a central pathology laboratory to further assess the histologic presence of PeIN. This is necessary because intraoperative specimen examination in MMS only evaluates the peripheral and margins and not the central tissue specimen. Systematic evaluation of the excised tissue by a central laboratory will ensure detection of any residual foci of PeIN in the excised specimens.

There will be six secondary outcomes in this study. The first will be partial response, characterized by 30% or greater decrease in diameter of the lesions from baseline after the treatment period. The second will be complete clinical response with persistent histologic disease, which is characterized by complete clinical resolution of the lesions from baseline, but with evidence of persistent histologic disease on post-treatment pathologic evaluation. The third will be progressive disease, characterized by 20% or greater increase in diameter of the lesion from baseline after the treatment period. The fourth will be stable disease characterized by neither sufficient increase or decrease in lesion size to constitute partial response or progressive disease after the treatment period. The fifth will be the incidence of recurrence during the follow-up period. The sixth will be time to recurrence during the follow-up period. To evaluate the partial response, progressive disease, and stable disease, we will photograph the pre-treatment lesion and

mark the circumference at 0º, 90º, 180º, and 270º around the lesion. We will then trace the lesion on a transparent paper and measure the diameter of the lesion along the longest axis and a perpendicular axis. After the treatment period plus one month, the lesions will be photographed again along with marking, tracing, and diameter measurement as at baseline. This will allow us to visualize any regression that occurred and quantify the magnitude of regression both in terms of maximum diameter and lesion area. To evaluate time to recurrence the clinicians will be monitoring the healing of the lesions after Mohs surgery and look for any signs of recurrence during the regularly scheduled follow-up appointments.

There will be three tertiary outcomes in this study. The first will be time to clearance of lesions during the treatment period. The second will be occurrence of secondary HPV-related lesions unrelated to the original lesion. The third will be the incidence of adverse events secondary to the interventions. These will be assessed at monthly follow-up appointments where the clinicians will conduct a detailed inspection of the genitalia to and assess any adverse events along with their severity according to Common Terminology Criteria for Adverse Events.[2]

In addition to these measurements and outcomes, all patients will have their initial biopsy specimens assessed for the presence and strain of HPV DNA with a laser capture microdissection (LCM)-PCR, which is a precise method of discerning genotypes for these tissue samples.[3] It is possible that not all institutions and hospitals that participate in this study have access to an LCM-PCR, so we will retain a part of the biopsy and bring them all to one location for assessment.

3.8 Blinding of Intervention

The clinicians and patients will be blinded to which medications are utilized. This will be made possible by creating identical tubes and removing any identifying information from the assistance of the manufacturers of the imiquimod cream. The only individuals who will be aware of treatment assignments will be central research personnel. Because imiquimod typically produces a strong local skin reaction, it is likely that both the patient and the clinician will form opinions about whether each patient is receiving imiquimod or placebo. This has the potential to compromise blinding. To account for this, we will separately assess at each monthly follow up visit what treatment the patient and clinical suspect they are receiving. This will be a multiple-choice data point, with three choices of imiquimod, placebo, and "I do not know." Regardless of the answer, patients will be instructed to continue to use their assigned treatment as directed for the duration of the study. Additionally, the central pathologist that will assess all Mohs specimens will be blinded to treatment category to eliminate a potential source of bias.

3.9 Assignment of Intervention

Randomization of the intervention and placebo will be made through a computer-program randomizer. Randomization will be stratified by two factors. First, randomization of subjects will be stratified by location, to ensure that even proportions of imiquimod and placebo are distributed between the different trial locations. Second, randomization will be stratified by PeIN subtype. Because erythroplasia of Queyrat has been observed to have a lower response rate to therapy than other types of PeIN, we will stratify to ensure that even proportions of erythroplasia of Queyrat are present in the

imiquimod and placebo arms of the study.[4] The order of which the intervention or placebo treatments will be determined prior to the start of the study, and the treatments will be delivered to the clinicians as soon as a patient is enrolled in the study.

3.10 Adherence

We will monitor adherence through a daily use diary that the patients in the study will keep and bring to scheduled monthly evaluations. In addition, weekly scheduled phone calls, texts, or emails will be sent during the treatment period to remind patients of the importance of compliance. The mode of communication will be determined by the preference of the patients. During these calls, texts, or emails, study personnel will ask if they are remaining adherent to the treatment regimen. We will note any non-adherence among the patients and have the research personnel provide guidance on whether the patients need to meet with their clinician for any unscheduled evaluations. Additionally, the patients will be meeting with their clinicians every 4 weeks during the treatment period to assess for side effects and clearance of the lesions. The patients will not receive communication from the research personnel during these weeks.

3.11 Monitoring of Adverse Events

It will be at the clinician's discretion to continue the treatment despite presence of side effects. They may also prescribe any other medications to alleviate side effects. If a patient endures more serious side effects to the imiquimod or placebo, it will be at their clinician's discretion to discontinue the treatment for 7 days. After one week, we ask that the clinicians resume the treatment at the same three days per week frequency as tolerated.

Since we anticipate that some of our patients will endure adverse reactions, we will allot one month after the treatment period for these side effects to resolve before MMS. This will allow for better outcomes and success during and after surgery.

3.12 Data Collection

The primary outcome of complete response will be assessed by the surgeons performing MMS. They will report to the research personnel of any histological presence of PeIN or invasive carcinoma in the recovered surgical specimens.

The secondary outcomes of partial response, progressive disease, and stable disease will be calculated from measurements obtained by the research personnel at each site. To ensure consistency, the research personnel will be provided training on how to properly measure lesions prior to the start of the study. The secondary outcome of incidence of recurrence and time to recurrence will be reported by the clinicians to the research personnel during the follow-up period. Similarly, the tertiary outcomes of time to clearance, incidence of side effects, and presence of secondary HPV-related lesions will also be evaluated by the clinicians and reported to the research personnel as they occur.

3.13 Sample Size Calculation

Using the only robust data available on imiquimod cream as treatment for PeIN, we will reference the 2017 Deen et al. study to find an effect size of 63%.[4] For the expected effect size in the control group, we will use a 2003 study by Swetter et al. who found that 24% of non-melanoma skin cancers, such as squamous cell carcinomas and basal cell carcinomas, regressed and showed no residual tumor after the initial biopsy without additional treatment.[5]

We will assume an alpha of 0.05 and a power of 80% with a 2-tailed hypothesis. Using these numbers, we calculated a sample size of 50 patients total, or 25 per treatment group. We will assume a 10% dropout during our study, so we will aim to recruit a total of 56 patients, with 28 in each treatment group. These calculations were made utilizing Power and Precision Software, and will be further detailed in Appendix B.

3.14 Analysis

This study will utilize an intention-to-treat analysis. Descriptive statistics will be as follows: PeIN classification (BP, BD, EQ, or not specified), PeIN type (undifferentiated or differentiated), HPV DNA, circumcision status, HIV status, currently taking immunosuppressive medications, history of cancer or inflammatory disease of the prepuce, history of genital warts, prior surgery on penis, smoking status, marital status, and BMI. Continuous variables will be reported as means (standard deviations) and categorical variables will be reported as frequencies (%). A p-value of <0.05 will be determined as significant. The primary and secondary outcomes of complete response, partial response, progressive disease, stable disease, and incidence of recurrence will be analyzed by a Chi-Squared test to compare the categorical variables. The secondary outcome of time to recurrence and tertiary outcome of time to clearance of lesion will both be analyzed by a Kaplan Meier curve. The tertiary outcomes of incidence of side effects and presence of secondary HPV-related lesions will be analyzed by a Chi-Squared test to compare the categorical variables.

To minimize confounding variables, we will utilize a multiple logistic regression analysis on all categorical outcomes, notably the primary outcome. Similarly, we will utilize a multiple linear regression analysis for all quantitative outcomes.

3.15 Timeline and Resources

This study will take place over two years once the study is approved by IRB at Yale and the respective IRB at all other hospitals and institutions where we intend to conduct this study. As previously mentioned, we will have an 8-month recruiting period. Once a patient is enrolled in the study, they will start a 3-month treatment period, followed by a 1-month period for side effect resolution, and end with a 12-month follow-up.

To properly conduct this study, we will require at least 3 research personnel with capabilities to travel within the New England and New York areas when new patients are recruited. This is to ensure the pre- and post-treatment measurements are conducted similarly irrespective of clinical site. We hope to recruit 2 investigators per site in either the urology or dermatology departments, and at least 1 investigator must be a Mohs surgeon. There will be one central pathologist to read all Mohs specimens. The principal and co-principal investigators will oversee the entire project and will conduct all outreach. They will be in contact with the clinicians and research personnel throughout the study to ensure that proper protocol is conducted.

Monetarily speaking, the research personnel will be paid for their work throughout the study. The patients, clinicians, and participating hospitals will not receive payment for their involvement. However, we intend to contact the manufacturers of the imiquimod cream with hopes to have the medication provided at no cost to the participants. Additionally, we hope to receive enough resources to reimburse the Mohs surgeons for surgery performed during the trial, and to provide a small monetary stipend to each patient upon trial completion. We also intend to reimburse any transportation fees

that the patients may encounter at their respective hospitals. This should provide some incentive to the patients as there will be no added costs to participating.

3.16 References

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Chapter 4: Conclusion

4.1 Advantages

This type study would be entirely novel to PeIN and has the potential to change how a clinician and their patient discuss the best course of treatment for their lesion. As mentioned previously, there are currently no RCTs for PeIN in literature. This study would also have the added benefit of being double-blinded, gather critical descriptive data such as HPV DNA, and standardize the treatment frequency among the patients. This study would also be multicenter providing more generalizability to the results.

Although the primary outcome of this study is studying the complete response of PeIN after treatment with imiquimod, the addition of post-treatment MMS for all patients has several benefits. This will provide more data on the success of MMS for PeIN and the recurrence afterwards. This data of this study could also compare the recurrence rates for the individuals who attain complete response with imiquimod and the non-responders in the placebo group who receive MMS. We realize this study is not properly powered to assess this, but the basis for the treatment of this disease is already so scarce that any data would be helpful. Simply speaking, this would provide more insight on the topical versus surgical debate for treatment of PeIN.

4.2 Disadvantages

Despite the novelty of this proposed study, there are several limitations and disadvantages to its design that are mostly unavoidable. This is a rare disease, thus why it constitutes the need for a multicenter approach and consecutive sampling. There will be an attempt to standardize the measurements and recordings among enrolled patients, but inconsistencies and differences in care are unavoidable. There is concern for patient

recruitment within the proposed eight-month timeframe. If 56 patients are enrolled in the study, this leaves little forgiveness for rejecting the null hypothesis if the effect size is not as great as previous studies have demonstrated. Patients will also be administering this medication themselves, and although we intend to educate them on proper application and contact them throughout the weeks to ensure proper adherence, it will ultimately be up to the integrity of the patient if he is applying the cream as prescribed. Due to the nature of imiquimod and the localized skin reaction it causes, it is likely that the clinicians and the patients will know whether they are receiving imiquimod or the placebo. This raises the concern for incomplete blinding and introducing a potential bias, however, this will be monitored with a brief multiple-choice question at their follow-up visits as mentioned previously. Additionally, there is always a concern of a loss to follow-up after the surgery, but outreach attempts will be made in an effort to minimize this event.

4.3 Clinical and Public Health Significance

Because PeIN is a rare disease, this study may not have a dramatic clinical or public health influence after its conclusion. However, the patients that are affected by this disease deserve more time and resources than previously portrayed in literature. This disease can be both physically and emotionally debilitating for the patients, and as a result, patients often delay treatment when they develop these lesions.[1] Conducting a study on this disease may also raise awareness of the effect that HPV has on men, as recent studies show that only a small proportion of men know that HPV can cause penile cancer.[2] Thus, increasing awareness of the link between HPV and penile cancer could elicit an increase in HPV vaccination uptake. Lastly, the methods utilized in this

proposed study could be used as a template for other rare diseases that are grossly understudied.

4.4 Future Directions

Imiquimod is only one of the many potential treatment options for PeIN. With regards to other penile-sparing strategies, a future study should implement many of the same methods proposed in our study but focus on 5-fluorouracil as an intervention. This topical cream has also shown promise in treating PeIN, and could be another option for men seeking treatment.[3] An ideal study for the treatment of PeIN would be a randomized controlled trial comparing imiquimod, 5-fluorouracil, and MMS. However, this type of study would require a lot of resources and likely be conducted nationally or internationally to recruit enough patients. A study such as this would be the gold standard to determine the most efficacious treatment option for PeIN.

4.5 References

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2. Zavaski ME, Hanske J, Löppenberg B, et al. Contemporary perceptions of human papillomavirus and penile cancer: Perspectives from a national survey. *Can Urol Assoc J.* 2019;13(2):32-37.
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Appendix A: Sample Consent Form

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT

YALE UNIVERSITY SCHOOL OF MEDICINE

Study Title: Topical Imiquimod as Primary Therapy Prior to Mohs Surgery for Penile Intraepithelial Neoplasia
Principal Investigator: Sean Christensen, MD, PhD; Joseph Miller, PA-SII
Funding Source: [*To be determined*]

Invitation to Participate and Description of Project

This is an invitation to participate in our research study designed to study the effects of topical imiquimod therapy for penile intraepithelial neoplasia. You are being asked to participate by your clinician because you have a diagnosis of penile intraepithelial neoplasia based on your biopsy results and are a suitable candidate to receive imiquimod as a treatment option. You will be a part of approximately 56 persons who will participate in this study within the New England and New York areas.

This information provided in this form should help you decide whether or not you would like to participate in this study. It will discuss the research protocol thoroughly so there are no surprises if you decide to participate, as well as discuss the risks and benefits and other alternative treatments. This form also provides information on how your medical records and personal information will be kept confidential, as well as how you can withdraw from the study if you so choose. Once you have a clear understanding on this study and would like to participate, you will be asked to sign this form. Any and all questions you have can be directed to the research personnel that provided you with this consent form.

Description of Procedures

Patients who decide to participate in this study will be randomly assigned to either receive 5% topical imiquimod cream, or a placebo cream. The treatment you are provided will be unknown to both you and your provider for the entirety of the study. This is in an effort to prevent any bias in care you receive from your clinician during this study. Before starting the treatment, someone from the research team will meet with you at an appointment to take photographs and measurements of the lesion.

You will be asked to apply the topical cream you are provided 3 times per week for 3 months. The cream will be applied for 12 hours at a time, then thoroughly washed off after the 12 hours have elapsed. During the treatment period, you will be asked to

complete a daily diary for any changes or symptoms that may arise. Someone from the research team will contact you weekly via your preferred contact method to serve as a reminder and to ensure you are tolerating the medication. Additionally, you will be meeting with your clinician every 4 weeks to monitor the progression of the skin lesion and how you are tolerating the medication. At these visits, you will be asked to complete 1 multiple choice question to see if you are aware of what treatment you were provided at the beginning of the study. After 3 months, you will have 1 treatment-free month to allow the medication time to potentially heal your skin lesion and for any potential adverse effects to reside. At the end of this month, someone from the research team will meet with you again at an appointment to take photographs and measurements of the skin lesion again. Once these measurements are recorded, all patients will undergo Mohs micrographic surgery to remove any remaining part of the lesion. This will also serve as a method of biopsy to ensure the entirety of the lesion is removed if it still remains.

After the surgery, you will meet with your clinician every 3 months for one year to monitor for signs of disease recurrence. After 1 year your participation in the study will end, and it will be at your clinician's discretion if they choose to continue following your progress.

If there are significant findings that develop over the course of the study that may affect your willingness to participate, you will be notified promptly and given the choice whether you'd like to continue or withdraw from the study. If the results of this research are published, there will be no identifying information in the publication.

Risks and Inconveniences:

The side effects of imiquimod include burning sensation, redness of skin, irritation, itching, tenderness, bleeding, crusting, loss of pigmentation, flu like symptoms, headache, and muscle aches. These side effects are generally well tolerated by patients, but there is a possibility that you may have to discontinue the therapy if the side effects are too intense. If you believe you are unable to tolerate the side effects, you can discuss this with your clinician at your follow up appointments or at the weekly reminders from the research personnel, and they can provide you advice for how to proceed with treatment.

The placebo cream should not cause side effects, but you should reach out to your clinician if side effects occur.

The Mohs surgery is a safe and minimally invasive procedure done in the office. This procedure will use local anesthetic and does not require general anesthesia. The length of the procedure is variable and may last a few hours. Complications are rare, and typically only include bruising or bleeding. These are both easily managed with compression and observation. Patients report different pain levels after the surgery, but in most cases the pain can be managed with typical over the counter medicines such as ibuprofen or acetaminophen. Opioids can be prescribed if the pain elicits its usage.

There is a risk that your personal health information is compromised during the study. However, this risk is minimal as all research personnel and clinicians involved with the study will undergo mandatory HIPAA (Health Insurance Portability and Accountability Act) training. You will be notified in the event that this occurs.

Benefits

The potential benefit of this study is the clearance of your skin lesion with minimal surgical intervention required. This study will also provide insight into the effectiveness of imiquimod for future patients and the design of this study may serve as a template for future research involving your disease.

Economic Considerations

If you participate in this study, you will receive a small stipend of $X at the end of the study. In addition, the treatment and the surgery will be at no cost to you, as well as any transportation cost for the follow-up appointments.

Treatment Alternatives/Alternatives

The alternatives for the treatment of this disease include topical 5-fluorouracil, cryotherapy, surgical excision, and laser excision. If you choose not to participate in this study, the chose in treatment will be at the discretion of your clinician.

Confidentiality and Privacy

Any personal identifying information including your name, birthdate, and medical record number will be deidentified after its acquisition in this study. Your information will remain deidentified indefinitely. The information at your screening visit and follow-up appointments will be input into your electronic medical record (EMR). This information will be accessible to any clinicians and healthcare professionals with EMR privileges, as well as health insurance companies.

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. Information will be kept confidential on a study-related forms, we will store all signed forms behind lock and key, and any electronic information will be kept on a password-protected computer and an encryption key. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity without your consent.

By signing this form, you authorize the use and/or disclosure of the information described above for this research study. The purpose for the uses and disclosures you are authorizing is for future parties who may need this study for research purposes.

Voluntary Participation and Withdrawal

You have no obligation to participate in this study and are free to decline participation. Your health care and payment for your health care will not be compromised. If you decline participation, you will not be included in the study described above and will not receive any compensation. You do not give up any of your legal rights by signing this form.

Withdrawing From the Study

If you participate in this study, you are free to withdraw at any point during the study. You will not need an explanation for that decision, and you may continue to receive care from your previously established health care team. Your decision to withdraw will not harm your relationship with the healthcare system for which you are receiving care.

If you wish to withdraw for any reason and at any time, you may reach out to a member of the research team or your clinician and request that you no longer wish to take part in the study.

Withdrawing Your Authorization to Use and Disclose Your Health Information

You are free to withdraw and relinquish your authorization to use and disclose your health information at any time. To do so, you can reach out to the principal investigators of this study. By withdrawing this authorization, no new health information will be gathered from you. However, all previously gathered health information will still be accessible for the remainder of the study as to not compromise the integrity of the study.

Questions

If you feel that any part of this form was unclear to you and would like further clarification, please feel free to reach out to the research team for questions. Do not feel pressured to participate in this study if you are uncertain, and please discuss your options with your clinician or the research team if you feel that is necessary.

Authorization

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject:_____

Signature:_____

Relationship:_____

Date:_____

_____ _____
Signature of Principal Investigator Date

or

_____ _____
Signature of Person Obtaining Consent Date

If you have further questions about this project or if you have a research-related problem, you may contact the principal investigator Dr. Christensen at any time.

If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

Appendix B: Sample Size Calculation

Sample Size Calculation Equations:

$$N_1 = \left\{ z_{1-\frac{\alpha}{2}} * \sqrt{\bar{p} * \bar{q} * \left(1 + \frac{1}{k}\right)} + z_{1-\beta} * \sqrt{p_1 * q_1 + \left(\frac{p_2 * q_2}{k}\right)} \right\}^2 / \Delta^2$$

$$q_1 = 1 - p_1$$

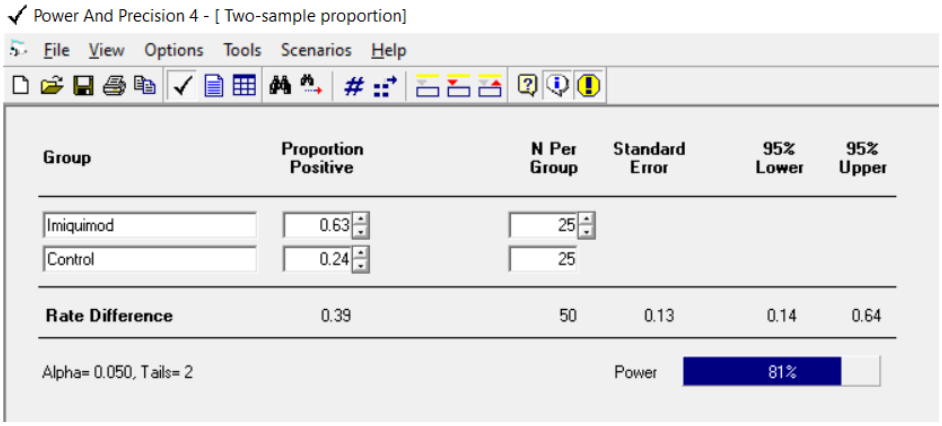
$$q_2 = 1 - p_2$$

$$\bar{p} = \frac{p_1 + k p_2}{1 + K}$$

$$\bar{q} = 1 - \bar{p}$$

p_1, p_2 = proportion (incidence) of groups #1 and #2
$\Delta = |p_2 - p_1|$ = absolute difference between two proportions
n_1 = sample size for group #1
n_2 = sample size for group #2
α = probability of type I error (0.05)
β = probability of type II error (0.2)
z = critical Z value for a given α or β
K = ratio of sample size for group #2 to group #1

Sample Size Calculation Software:



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