Review

Development challenges for carcinogenicity risk assessments of topical drugs

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(Received October 25, 2021; Accepted October 25, 2021)

ABSTRACT — The nonclinical safety package to support development and approval of drugs intended to be administered by topical application generally follows International Council for Harmonisation multidisciplinary 3 (ICH M3) and topic specific safety (ICH S) guidances. However, some aspects of topical drug development may require case-by-case determination of nonclinical safety strategies. The necessity to conduct a dermal rodent carcinogenicity study is one such example that is not considered an obligate component of a nonclinical safety data package for drug approval. While absence of systemic exposure, as stated in ICH M3, is a primary reason to forego a dermal carcinogenicity assessment, there may also be other factors for consideration in determining the need for a life-time carcinogenicity study by dermal route to aid in the overall human cancer risk assessment. We therefore reviewed nonclinical carcinogenicity data packages from drugs approved by the FDA or PMDA over a ~25 year time period to evaluate outcomes of oral versus topical carcinogenicity studies and to understand their utility for informing the overall human risk assessment. We also discuss various other properties of topical small molecules that could impact the decisions to conduct a dermal life-time rodent carcinogenicity study. Collectively, the need to conduct 2-year dermal carcinogenicity studies in rodents should be determined case-by-case and consider scientific factors such as existing systemic toxicity and carcinogenicity study data, anticipated drug exposures in skin, skin evaluation from the chronic minipig toxicity study, and genetic toxicity profile.

Key words: Topical carcinogenicity, Aneugen, Skin concentration, Topical drug

INTRODUCTION

Multiple factors guide a strategy to design and develop medicines intended to be administered by the topical route. Typically, a primary motivation is that the clinical condition or disease intended to be treated manifests in skin and applying a drug directly to the site of interest has the highest likelihood of achieving clinical benefit. It is not uncommon for topical drugs to be reformulations of a compound previously approved or also in clinical development as a systemically bioavailable (e.g., oral or parenteral administration) drug, and the development of novel or reformulated topical drugs may be intended to reduce systemic safety liabilities, maximize concentrations of drugs in the skin, or to increase patient use and compliance (Feldman *et al.*, 2007; Florek *et al.*, 2018; Tieppo

Francio *et al.*, 2017).

The nonclinical safety data packages required to support clinical development and marketing authorization of topical small molecule drugs are generally like those anticipated to be administered by oral and/or parenteral routes. General toxicology, safety pharmacology, genetic toxicity, developmental and reproductive toxicity, and phototoxicity assessments, for example, are usually required to support clinical testing of topical drugs and follow principles outlined in ICH multidisciplinary and discipline specific safety guidance documents. There can also be a need to conduct other specialized studies to characterize skin-related risks for a topical drug. These types of studies, for example, could include *in vitro* assessments to characterize potential risks related to skin sensitization (OECD, 2018a, 2018b, 2020) or ocular irri-

tancy (OECD, 2009).

One of the final nonclinical studies conducted to support a marketing authorization application for a small molecule is a rodent 2-year carcinogenicity study. This ‘life-time’ study in a rodent is the pivotal nonclinical study for most new drug applications used to describe the potential for a drug to have cancer-inducing potential in humans. However, the necessity to conduct a 2-year carcinogenicity study is not always a straightforward question to answer and a critical evaluation of the need to conduct such studies as a requisite component of overall nonclinical safety packages should be considered on a case-by-case basis (Sistare *et al.*, 2011). An ICH S1B Expert Working Group effort is ongoing to understand when 2-year rodent carcinogenicity studies provide meaningful additional information to support the overall human carcinogenicity risk assessment for systemically bioavailable drugs, compared to when such a study would provide limited additional value to the overall assessment.

It is established that agents possessing certain characteristics, such as endocrine disruption, immune suppression, chronic toxicity, and genotoxic activity, are more likely to possess cancer promoting properties (Monro, 1993). Compounds that contain one or more of these properties often have assumed human risks that would not be further informed by rodent carcinogenicity study results excluding questions related to possible species relevance. Additionally, topically applied drugs without characterized neoplastic risks and/or limited systemic exposure may also have questions around the value in completing rodent carcinogenicity studies.

In this brief review, we have evaluated the pharmacology summary packages and/or labels from 23 topical drug approvals by the United States Food and Drug Administration (FDA) spanning from 1997 through 2020. We additionally have reviewed two recent nonclinical approval packages from topical drugs by the Japan Pharmaceuticals and Medical Devices Agency (PMDA). Collectively, the nonclinical packages for the approved topical drugs that were reviewed highlight that nonclinical carcinogenicity data packages can vary significantly and that conduct of a topical carcinogenicity study should not be viewed as an obligate study required for the approval of all small molecule topical drugs. We further discuss considerations that may be factored on a case-by-case basis to evaluate the necessity to conduct a rodent carcinogenicity study to support topical drug authorization. Proactively identifying scenarios for topical drug development that would not require the need to conduct 2-year studies can help to reduce overall animal usage in drug development programs while maintaining high qual-

ity human safety assessments.

CONSIDERATIONS FOR DETERMINING NEED FOR TOPICAL CARCINOGENICITY STUDY

Physiologic similarity or differences of skin between animals and humans, drug exposures in the viable and/or proliferating compartment of skin, and genotoxicity profile are important factors in considering the needs for a topical carcinogenicity study.

Nonclinical species skin relevance

Animal-to-human translational relevance is well accepted to be a challenge for understanding human risks derived from animal studies and safety factor adjustments for sensitivity differences between a test species and humans may be incorporated into safety assessments when human translation is uncertain (Dorne and Renwick, 2005). While the rodent (rat, mouse) is a key test system for nonclinical testing of systemically bioavailable drug candidates prior to and during human clinical development, its skin is well known to have significant morphologic and physiologic differences compared to humans (Bartek *et al.*, 1972; Wei *et al.*, 2017).

Unlike the rodent, the minipig has comparatively similar skin morphology and physiology compared to human and is used as the species of choice for dermal evaluation by topical administration in nonclinical toxicity studies (Mortensen *et al.*, 1998; Stricker-Krongrad *et al.*, 2017). Skin in human and minipig is comprised of a relatively thick epidermis and dermis compared to the rodents (Wei *et al.*, 2017). Rodents by contrast have a very dense hair coat with up to ~300–650 hair follicles per cm² while the hair follicle density in human and minipig is ~11 per cm² (Stricker-Krongrad *et al.*, 2017). The primary barrier protection for skin absorption in humans and minipigs is mediated via the outermost layer of the epidermis, the stratum corneum (SC), which is made up of multiple layers of tightly compacted keratin and horny scales derived from dead keratinocytes (Mortensen *et al.*, 1998). Like the overall epidermis and dermis, the SC in rodents is also thinner than humans or minipigs (Mortensen *et al.*, 1998; Wei *et al.*, 2017). Complete epidermal turnover occurs in normal human and minipig skin is approximately every 4–8 weeks, while the turnover rate in mice is about 1-week (Koster, 2009; Stricker-Krongrad *et al.*, 2017). Given the high density of hair follicles in rodents and thin epidermis it is reasonable to conclude that in rodents the fur coat provides significant barrier protection, and not surprisingly rodent skin itself is generally more permeable to topically applied drugs than human

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and minipig (Bartek *et al.*, 1972; Fujii *et al.*, 1997). This is particularly noteworthy when considering that topical rodent studies are conducted by removal of the hair coat so that a test article can be applied directly to the skin. Therefore, it is not surprising that systemic bioavailability by topical application is often higher in rodents compared to human or minipig and can limit the utility and interpretability of rodent models for topical safety evaluations. This is particularly important for topical carcinogenicity studies as skin findings could be difficult to interpret regarding human relevance given the numerous physiological and anatomical species differences.

Collectively, the multifactorial differences between rodent and human skin emphasizes the value of the minipig test system for characterizing safety liabilities of a topical drug candidate in the skin. As discussed in the ICH S1A guideline, a potential cause for concern for carcinogenicity risk (e.g., preneoplastic lesions) can be assessed from routine repeat-dose chronic dermal toxicity studies. Since the epidermis in the minipig turns over approximately once every month (Stricker-Krongrad *et al.*, 2017) a chronic repeat-dose dermal toxicity study of 9-months in duration allows for at least 8 epidermis remodeling events. A careful review of the microscopic evaluation of skin from these minipig studies should be incorporated into the overall carcinogenicity risk assessment. A lack of preneoplastic findings in minipig skin following chronic topical administration may provide supportive information that would mitigate the need to further characterize a topical drug in a 2-year rodent dermal carcinogenicity study.

Drug concentrations in skin

A considerable challenge of positioning nonclinical safety skin findings for human risk deals with the prediction of drug concentrations within the skin resulting from clinical use. Systemic toxicity margins are usually derived by measuring plasma drug concentrations as a surrogate for exposure in other tissues (Lehr, 2011). Measurement of plasma drug concentrations in animals and humans with validated bioanalytical methods allows for precise understanding of anticipated concentrations of a compound that would carry a safety concern compared to those achieved when a drug is administered at clinically relevant doses. This information is an important contributor for determining starting doses in first in human clinical trials and to contextualize risks throughout development (Shen *et al.*, 2019).

For topically administered drugs, however, there are currently no standard validated approaches for refined measurements in specific skin compartments. Due to this,

safety margins for topical studies are usually not calculated based on exposure but rather are calculated on a dose per unit area (e.g., mg/cm²/dose). This reliance on dose margin based calculations underscores the importance of using a test system that is comparatively similar to human, like the minipig, for dermal safety assessments.

Multiple approaches have been described to measure drug concentrations in the skin. A common method is based on homogenization of skin biopsies. While this method can be conducted using standard experimental and analytical techniques it is usually confounded by artifactually high measured drug concentrations introduced via sample collection (Eirefelt *et al.*, 2020; Handler *et al.*, 2021; Surber *et al.*, 1993; Trotter L., 2017). Additionally, a full biopsy homogenate will principally only represent the drug located in the SC or superficial epidermal layers which are nonviable or non-proliferative and rapidly shed, respectively, and therefore are of limited value for safety assessments for threshold mediated mechanisms of toxicity (Hernández *et al.*, 2013; Olaharski *et al.*, 2009). More refined methods for addressing drug concentrations in dermal and or epidermal compartments include microdialysis, open flow microperfusion (OFM), mass spectroscopy imaging, and laser capture microdissection (LCM) approaches (Bodenlenz *et al.*, 2017; Bonnel *et al.*, 2018; Herkenne *et al.*, 2008; Hollingshead *et al.*, 2021).

Given the high amount of active drug that is applied to the skin by topical application, a nominal skin concentration assuming 100% absorption would be extremely high throughout the skin (e.g., > 1 Molar). This phenomenon, however, is not observed in clinical practice for pharmaceutical drugs since their physicochemical properties are not highly favorable for high skin absorption and steep concentration gradients are observed across the skin. Human and/or minipig concentrations of various drugs in the dermis or epidermis assessed by methods such as OFM, mass spectroscopy imaging, and LCM have illustrated that drug concentrations in viable skin are a fraction of that found in the SC. In humans, for example, studies with OFM have measured average dermal concentrations of an antiviral (acyclovir) and phosphodiesterase inhibitor (LEO 29102) ranging from ~10 to 1000 nM when applied topically (Bodenlenz *et al.*, 2020; Eirefelt *et al.*, 2020). Likewise, in a pig OFM study, dermal interstitial fluid concentrations of the Janus kinase inhibitors tofacitinib and LEO 27319A were measured at ≤ 100 nM (Handler *et al.*, 2021). A study in minipigs using LCM-based analytical approaches with a topically applied kinase inhibitor demonstrated a very steep concentration gradient within the epidermis, with > 50-fold lower concentrations in proliferative epidermal stem cells (i.e., stra-

Fig. 1. Drug concentrations in the epidermis and dermis of topical drugs have been shown to follow a steep concentration gradient from the site of application through the epidermal and dermal layers of the skin. For many topical drugs the active pharmaceutical ingredient is retained in the non-viable outer SC, with only a small fraction reaching the proliferative basal epidermis or viable dermis. While molar (M) concentrations of active drug can be detected in the SC, reports for multiple drugs in human and/or minipig skin have detected substantially lower (nM to μ M) concentrations at skin locations more relevant for carcinogenicity risk, such as the epidermal stratum basale.

tum basale) compared to the SC, and similarly low concentrations of drug in the dermis as reported with other compounds by OFM (Hollingshead *et al.*, 2021). This skin epidermal concentration gradient in tissue collected by the LCM method is similar to that reported for the topical calcineurin inhibitor, pimecrolimus, by quantification of radiolabeled compound (Hultsch *et al.*, 2005).

Concentrations of drug in the proliferative epidermal stem cells of the stratum basale and dermis are likely most relevant for understanding potential dose-related carcinogenic risk. As described above, and illustrated in Fig. 1, the concentrations of many topical drugs in the dermis and basal epidermis are present in the nanomolar and low micromolar range. These are concentrations that are often achieved in plasma in the context of an oral toxicity study. Therefore, it is possible that concentrations of drug achieved in the skin by oral toxicity studies in rodents for compounds with suitable volumes of distribution may be sufficiently high to cover the anticipated exposure in humans by topical application (Rodgers *et al.*, 2005; Rodgers and Rowland, 2006). In these cases, an appropriately conducted oral carcinogenicity study

may adequately support a dermal carcinogenicity risk evaluation without need to conduct an additional 2-year dermal carcinogenicity study consistent with guidance from ICH S1A.

Genetic toxicity profile

All small molecule drug development candidates will be evaluated in a battery of *in vitro* and *in vivo* assays, which are intended for hazard identification of a compound's ability to induce gene mutations, structural and/or numerical chromosomal aberration (ICH S2(R1)). The test battery usually consists of the bacterial Ames assay to identify gene mutagens, a mammalian test for clastogenicity and aneugenicity and an *in vivo* test for chromosomal aberrations in the blood or bone marrow of exposed animals.

Compounds that induce gene mutations in the bacterial Ames assay have a high likelihood of being carcinogens and are usually removed from drug development consideration unless the indication justifies. Similarly, clastogens without mechanistic information indicating low risk for genotoxicity are rarely progressed to clinical development. A special case is usually made for aneugens that are not expected to interact with DNA directly, but impact structures involved in the accurate distribution of chromosomes during mitosis (Aardema *et al.*, 1998). Aneuploidy inducing agents do generally not induce gene mutations but the loss or gain of whole chromosomes via threshold mechanisms (Elhajouji *et al.*, 2011, 1997, 1995). The best described mechanism for aneuploidy induction is probably tubulin binding, which causes mitotic spindle abnormalities leading to chromosome segregation errors and aneuploidy induction (Lynch *et al.*, 2019). In addition, kinase inhibitors in drug development are frequently shown to be *in vitro* aneugens via off-target inhibition of kinases involved in the regulation of mitotic processes like aurora kinase B (Sasaki *et al.*, 2020). Both, tubulin binding and aurora B inhibition are mechanisms that are highly toxic to dividing cells and induce very frequently toxicities in the gastrointestinal tract and in the bone marrow (Bates and Eastman, 2017; Bavetsias and Linardopoulos, 2015; Falchook *et al.*, 2015).

Systemically applied drugs with aneuploidy findings are usually risk managed using safety margin arguments comparing the no effect level concentration in the *in vivo* micronucleus studies with the systemic exposure in humans.

For topically applied compounds, the determination of safety margins is somewhat more complicated. Since aneuploidy can only be detected in dividing cells, aneuploidy induction in the skin focuses mainly on the divid-

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ing keratinocytes of the stratum basale. As indicated above, only a small percentage of a drug applied topically will likely reach the stratum basale and the accurate determination of compound concentration in this compartment has been rather difficult. In addition, no validated *in vivo* aneugenicity assessment method for human or minipig skin exists, adding to the difficulty for the evaluation of drug candidates. For this reason, we have assessed the suitability of the reconstructed skin micronucleus assay in the EpiDerm™ model for the *in vitro* assessment of topically applied aneugenic compounds (Schuler *et al.*, 2021). In addition, we looked for mechanistic key event biomarkers like increases in the mitotic index and polyploidy induction that could be characteristic for aneugenic effects in the dividing keratinocytes. While the EpiDerm™ system was very sensitive for micronucleus and biomarker induction, no significant difference in the dose-response was observed whether the dose was applied topically or has direct access to the cells of interest when dosed via the media. Based on these results, it became clear that a significant limitation of the *in vitro* EpiDerm™ appears to be the absence of barrier integrity to limit compound absorption like what would be expected in human and minipig skin, especially when acetone is used as a solvent. This is further supported by an *in vivo* experiment in the same publication that applied significantly higher doses of aneugens, when compared to the *in vitro* EpiDerm™ experiments, topically to the skin of minipigs and did not show evidence of the induction of key event biomarkers (Schuler *et al.*, 2021). These disparate results highlight the known importance of the barrier function of skin that likely does not allow adequate concentrations of even potent aneugens to reach the stratum basale. Additionally, colchicine has been tested in human clinical trials using up to 1% or 10 mg/mL, concentrations greatly exceeding those resulting in positive MN response in the EpiDerm™ model, for actinic keratoses and recalcitrant psoriasis for up to 12 weeks with no changes in the skin or systemic toxicity. (Akar *et al.*, 2001; Faghihi *et al.*, 2016; Kaidbey *et al.*, 1975). The overall weight of evidence indicates that aneugens, even at high doses, are unlikely to be aneugenic in human skin presumably due to its barrier function preventing sufficient exposure to the stratum basale.

Lastly, while aneuploidy is regarded as a hallmark of cancer, its role in the carcinogenic process is complex. A recent International Workshop on Genotoxicity Testing reviewed the role of aneuploidy in cancer biology; cancer risk associated with constitutive aneuploidy; rodent carcinogenesis with known chemical aneugens; and chemotherapy-related malignant neoplasms (Tweats

et al., 2019). The expert working group concluded that the aneugens reviewed that induce cancer in humans and animals all possess other carcinogenic properties, such as mutagenicity, clastogenicity, cytotoxicity, organ toxicities, hormonal, and epigenetic changes which likely account for, or interact with aneuploidy, to cause carcinogenesis. Tubulin-disrupting aneugens that do not possess other properties linked to carcinogenesis, were not carcinogenic in rodents (EMA, 2001; IARC, 1981a, 1981b; Longstaff and von Krogh, 2001; Takeda, 2017). In fact, colchicine significantly reduces the incidence cancer in a 12-year cohort study in male gout patients (Kuo *et al.*, 2015).

Based on the overall weight of evidence, we conclude that for aneugens that do not show cytotoxicity in the skin, and do not possess other confounding properties like clastogenicity or mutagenicity, there should be no need for topical carcinogenicity studies.

REVIEW OF MARKETED TOPICAL DRUG NONCLINICAL CARCINOGENICITY PACKAGES

To examine the nonclinical strategies used to characterize carcinogenicity risk for FDA and PMDA approved topical drugs we reviewed available summary information from the Drugs@FDA database (www.accessdata.fda.gov/scripts/cder/daf/) and for PMDA Drug Review reports (www.pmda.go.jp/english/review-services/reviews/approved-information/drugs/0001.html) available in English. We focused on topical drugs that were granted marketing authorization within the past 25 years by the FDA intended for longer than intermittent clinical use to both review the scope of nonclinical carcinogenicity packages, but also to determine if trends in strategy for assessing such risks have evolved during that time. We also reviewed the summary documents for two (sirolimus and delgocitinib) recently approved topical drugs in Japan. It should be noted that summary review documents contain variable amounts of detail for study types, results, and overall interpretations. Therefore, this assessment is not intended to be a review of all topical drugs approved during this timeframe, and data summarized in Table 1 should be interpreted with the understanding that information available to review in these documents are not comprehensive and can contain inconsistencies. Nevertheless, a holistic analysis of carcinogenicity packages across these approved products highlights trends and provide useful case studies for overall weight of evidence considerations of scenarios for the value of topical carcinogenicity studies.

The carcinogenicity packages for 23 topical drug products approved between 1997 and 2020 are listed in

Table 1. Carcinogenicity packages for approved topical drugs.

Drug	Topical Approval	Oral Carcinogenicity		Topical Carcinogenicity		Photocarcinogenicity	
		Type	+/-	Type	+/-	Type	+/-
Minoxidil	1997	2-year rat	-	2-year rat	⁺ (adrenal, preputial gland)	1-year hairless mouse	-
		2-year mouse	⁺ (lymphoma)	2-year mouse	-		
Diclofenac	2000	2-year rat	-	2-year mouse	-	1-year hairless mouse	+
		2-year mouse	-				
Tacrolimus	2000	2-year rat	-	2-year mouse	⁺ (lymphoma)	1-year hairless mouse	+
		80-week mouse	-				
Pimecrolimus	2001	Two 2-year rat	⁺ (thymoma)	2-year rat	⁺ (thyroid)	1-year hairless mouse	⁺ (vehicle-related)
		2-year mouse	⁺ (lymphoma)	2-year mouse	-		
Azelaic acid	2002	None		6-month Tg.AC and 2-year dermal mouse (post marketing)	-	None	
Dapsone	2005	2-year rat	-	6-month Tg.AC mouse	-	1-year hairless mouse	-
Desonide	2006	None		6-month Tg.AC mouse (post marketing)	⁺ (skin) ⁺ <i>in vitro</i> mouse lymphoma assay (potential mutagen)	None	
Imiquimod	2010	2-year rat	-	2-year mouse	⁺ (liver)	1-year hairless mouse	-
Adapalene	2010	2-year rat	⁺ (adrenal, pancreas, thyroid)	2-year mouse	-	None	
Ivermectin	2012	None		None		None	
Igenol mebutate	2012	None		None		None	
Luliconazole	2013	None		None		None	
Brimonidine	2013	2-year rat	-	2-year rat	-	1-year hairless mouse (recommended prior to ICHM3 revision)	-
		2-year mouse	-				
Ivermectin	2014	2-year rat	⁺ (liver)	2-year mouse	-	None	
efinaconazole	2014	None (waiver)		2-year mouse	-	None	
Tavaborole	2014	2-year rat	-	2-year mouse	-	None	
Crisaborole	2016	2-year rat	⁺ (uterus)	2-year mouse	-	None	
Oxymetazoline hydrochloride	2017	6-month transgenic mouse (waiver for 2-year, would not be tolerated based on 6-month tox)	-	None		None	
Ozenoxacin	2017	None		None		None	
Trifarotene	2019	2-year rat	-	2-year mouse	-	None	
Sirolimus (Japan)	2018	2-year mouse	⁺ (leukemia, liver)	Mid-term mouse skin carcinogenicity	-	None	
		2-year rat	⁺ (testes)				
Minocycline	2019	2-year rat	⁺ (thyroid)	None (Waiver)		None	
		2-year mouse	-				
Delgocitinib (Japan)	2020	2-year rat	⁺ (thymus, testes, pancreas, subcutis)	2-year mouse	-	None	
Clascoterone	2020	None		2-year rat	-	None	

- = no test article-related tumor findings

SBA References: (Pharmaceuticals and Medical Devices Agency, 2014; Pharmaceuticals and Medical Devices Agency, 2018; Pharmaceuticals and Medical Devices Agency, 2019; US Food and Drug Administration, 1997; US Food and Drug Administration, 2000a; US Food and Drug Administration, 2000b; US Food and Drug Administration, 2001; US Food and Drug Administration, 2002; US Food and Drug Administration, 2005; US Food and Drug Administration, 2006; US Food and Drug Administration, 2010a; US Food and Drug Administration, 2010b; US Food and Drug Administration, 2012a; US Food and Drug Administration, 2012b; US Food and Drug Administration, 2013a; US Food and Drug Administration, 2013b; US Food and Drug Administration, 2014a; US Food and Drug Administration, 2014b; US Food and Drug Administration, 2014c; US Food and Drug Administration, 2016; US Food and Drug Administration, 2017a; US Food and Drug Administration, 2017b; US Food and Drug Administration, 2019a; US Food and Drug Administration, 2019b; US Food and Drug Administration, 2020)

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Table 1. The overall breadth of these study packages varies but generally reflects a trend in more recent approvals for lower number of carcinogenicity studies. The absence of photocarcinogenicity studies after ~2013 is expected since reduction of this study type was recommended in the 2009 ICH multidisciplinary guidance revision (ICHM3 (R2), Note 6). The topical products listed in Table 1 that were approved between 1997 and 2001 (minoxidil, diclofenac, tacrolimus, and pimecrolimus) completed carcinogenicity study packages inclusive of oral, topical, and photocarcinogenicity studies. These contained 2-year oral carcinogenicity studies in rats and mice, a 2-year topical carcinogenicity study in mice (rat and mouse for minoxidil), and a mouse photocarcinogenicity study for a total of 4-5 carcinogenicity studies to support each products marketing application. The large number of studies conducted for these drugs likely are reflective of a combination of two elements: 1) design and execution of studies prior to ICH S1 guidance implementation and/or 2) prior or concurrent development of the active pharmaceutical compound for systemic treatments. The total number of carcinogenicity studies summarized to support a topical drug approval after 2001 typically range from 0 to 3. The rationale for not conducting carcinogenicity studies for some products was based on limited duration of use (e.g., lice (ivermectin), actinic keratosis (ingenol mebutate), or infection (ozenoxacin)) or low systemic bioavailability combined with absence of preneoplastic effects in chronic minipig toxicity studies (e.g., antifungal (luliconazole)) (US Food and Drug Administration, 2012a, 2012b, 2013a, 2017b). Ivermectin, for example, did not submit any carcinogenicity studies to support its use for the treatment of lice in 2012 based on its limited duration of use clinically, but its 2014 approval for use in treating Rosacea included submission of carcinogenicity data from 2-year oral rat and 2-year topical mouse studies (US Food and Drug Administration, 2014c).

It is noteworthy that for all compounds summarized in Table 1, there are no examples of test article-related skin tumors occurring at the site of drug application in 2-year topical rodent carcinogenicity studies. It is possible that this selection of drugs is skewed towards favorable profiles since it only represents approved drug products. However, it does also highlight the possibility that the rodent may not be sensitive for detecting skin carcinogenicity risk, or that skin is not a sensitive target in these studies, particularly since other types of tumors (e.g., liver, adrenal, thyroid, etc.) were observed in some topical studies in the absence of skin tumors. The only example that detected positive evidence for test article-related skin tumors was from a Tg.AC transgenic mouse model

for desonide, but the relevance of that finding to human was considered uncertain and results confounded by the possible mutagenic activity identified *in vitro* (US Food and Drug Administration, 2006). Additionally, while the Tg.AC mouse carcinogenicity assay has been utilized as an alternative model for some products the relevance of this model to human risk is unclear and it is no longer used in the United States (Jacobs and Brown, 2015).

Interestingly, minoxidil, tacrolimus, and imiquimod each conducted oral and topical rodent carcinogenicity studies, but only identified drug-related tumors in the topical studies at locations outside of the application site. While some of these observations could be related to strain sensitivities to tumors (Friedrich and Olejniczak, 2011) it is also likely that tumors were observed in topical studies but not oral studies for these drugs due to systemic exposure differences. Tacrolimus, for example, noted lymphoma in the 2-year topical mouse, but not in the 2-year oral rat or 80-week mouse carcinogenicity studies. The observation of lymphoma in the topical mouse study, however, occurred at higher plasma levels than achieved in either of the oral studies and at large exposures relative to the clinically relevant human levels. In fact, the plasma exposures at the no observed effect level for tumors in the topical and mouse oral carcinogenicity studies were almost identical (US Food and Drug Administration, 2000a). Similarly, the finding of imiquimod-related liver tumors in the 2-year mouse topical carcinogenicity study occurred at higher plasma exposures than those achieved in the 2-year oral rat study (US Food and Drug Administration, 2010b). These cases serve as cautionary examples of the point introduced earlier regarding the high permeability of drugs when applied to in rodent skin compared to human and minipig.

Conversely, increases in delgocitinib-related tumors (thymus, testes, pancreas, subcutis) were identified in the 2-year oral rat carcinogenicity study, but not the 2-year topical mouse study. While species differences could account for some of these observations all delgocitinib-related tumor findings were observed at doses achieving higher plasma exposures than those achieved in the dermal mouse study. Delgocitinib is an interesting case study since higher incidence of epidermal hyperplasia was observed in the chronic dermal minipig toxicity study in delgocitinib-containing formulations (Common Tabulated Document, Section 2.6), but the 2-year dermal mouse carcinogenicity study was negative for increased incidence of compound-related skin tumors (Pharmaceuticals and Medical Devices Agency, 2019). This minipig microscopic finding could be interpreted as a signal for neoplastic risk, however, it is noteworthy that incidence of epider-

mal hyperplasia was similar to control animals following completion of a 1-month recovery phase. This recovery data supports that the epidermal hyperplasia at the end of the dosing phase was not an indicator of neoplastic risk, but rather a normal physiologic response to skin irritation (Toussaint *et al.*, 1999), and is a reminder of the importance of incorporating expert pathology interpretation in the overall risk assessment for a product.

Taken together, a review of 23 drugs approved for topical application show a declining trend toward conduct of topical rodent carcinogenicity studies, with test article associated tumors related to systemic exposures not local application site effects. The absence of identified skin risks in rodent carcinogenicity studies highlights the value of evaluating skin effects in more human relevant minipig chronic toxicity studies to determine if any human risks are present.

CONCLUSION

While a complete nonclinical safety package for topical drugs is generally similar to other routes of administration there are considerations that could necessitate the conduct of additional studies or elimination of others. We have summarized above some of the main considerations that should be evaluated as part of the overall assessment of skin carcinogenicity risk of a topical product and suggest a decision process for determining if a 2-year topical rodent carcinogenicity study would add to the overall carcinogenicity risk assessment. The schematic in Fig. 2 is focused on topical drugs that are intended for longer than intermittent clinical use and have some systemic exposure liabilities since as mentioned earlier there are examples of topical drugs that did not require any studies based on these criteria. Additionally, this decision process may not be applicable if inactive ingredients in a novel drug product are unprecedented.

Fundamentally, the decision process outlined in Fig. 2 considers that if skin exposures are below levels of concern and within ranges achieved by oral studies in rodents then there is limited utility in conducting additional topical carcinogenicity studies in rodents. It also incorporates in the decision process an assessment of skin findings from the minipig chronic toxicity study and overall genetic toxicity risk profile to determine if any signal for concern is present. While there are without doubt circumstances that will necessitate the need to conduct rodent carcinogenicity studies based on absence of comprehensive supportive information, these studies should not be viewed as obligate when sufficient supportive information already exists for the overall weight of evidence risk

Fig. 2. A set of decision criteria are outlined to determine if a topical drug in clinical development should consider conducting a topical rodent carcinogenicity study or if it is unlikely to add to the overall human risk assessment. The supportive information that should be considered should include, but not necessarily limited to, existing systemic toxicity and carcinogenicity study data, anticipated exposures in skin and plasma, relative risk based on skin evaluation in a chronic minipig toxicity study, and genetic toxicity risk profile.

assessment. The overarching principals guiding this dermal carcinogenesis study decision process do have precedent based on the summary approval packages for minocycline (US Food and Drug Administration, 2019b) and luliconazole (US Food and Drug Administration, 2013a), both of which received dermal carcinogenicity study waivers due to absence of neoplastic concern from other studies, with particular focus on the chronic minipig results. In summary, conducting 2-year dermal carcinogenicity studies in rodents only when scientifically justified will also aid to achieve the 3Rs principles for reduction of unnecessary animal testing without compromising safety and effectiveness for topical drug products.

Conflict of interest---- The authors of this manuscript were employed by Pfizer, Inc. during the conduct of this work and are shareholders of Pfizer Inc. stock.

Abbreviations

International Council for Harmonisation (ICH), Food and Drug Administration (FDA), Pharmaceuticals and Medical Devices Agency (PMDA), stratum corneum (SC), open flow microperfusion (OFM), laser capture microdissection (LCM)

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