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Review Article

Desmocollin-3 and Cancer

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Abstract

Desmocollin - 3 (DSC3) is a trans-membrane protein, expressed in stratified epithelium. During carcinogenesis it is over-expressed in a few cancers of epithelial origin and down-regulated in others. DSC3 expression is associated with improved survival compared to DSC3 negative in a limited clinical trial data. Currently no treatment available for DSC-3 expressed cancer. Mycidac-C (mycobacterium-w) is a world's first active immune therapeutic agent targeting DSC3. It is a pure potent Th1 response enhancer.

As a mono therapy it delays progression of tumor and works synergistically with chemotherapy. In humans it improves overall survival in NSCLC without adding any additional systemic side effects. The improvement is pronounced in Squamous NSCLC compared to non-squamous NSCLC. As an intradermal mono therapy it is found useful in the management of Non Muscle Invasive Bladder Cancer for prevention of recurrence and avoiding intravesical instillation. Thus Mycidac-C appears to be a novel evolving therapy for DSC3 expressing tumors.

Keywords: Desmocolin-3; Cancer; Mycidac-C (Mycobacterium-w)

Key messages

- DSC-3 is novel target for developing novel therapies.
- DSC-3 expression is seen in NSCLC, Bladder cancer, Melanoma, Head and Neck Cancer, Prostate cancer and optic neuritis.
- Cadi-05 (Mycidac-C) an world's first active immunotherapy targeted for DSC3 offers a new treatment option in management of DSC3 expressing tumors.

Introduction

Desmocollin-3 (DSC3) is a member of the cadherin superfamily (transmembrane proteins) of calcium-dependent cell adhesion molecules and a principle component of desmosomes [1-5]. Desmosomes provide membranous anchors for intermediate filament cytoskeleton and connect intracellular intermediate filaments to the cell surface to mediate strong cell–cell adhesion [3,4,6]. and participate in maintenance of normal tissue structure in the epidermis [1].

Of three desmocollins, Desmocollin-2 is expressed in all desmosome - bearing tissues, while Desmocollin - 1 and Desmocollin - 3 are expressed, mainly in stratified squamous epithelia [3]. Desmocollin - 3 is mainly observed in the basal and immediate suprabasal layers [3,7-9]. DSC3 is also expressed in non keratinizing epithelium like buccal mucosa, cervix, esophagus, tongue, trachea etc [10].

Figure 1 shows DSC3 expression relative to human mammary epithelium cells (HMECs) as assessed by real-time quantitative RT-PCR [3]. In humans all demosomal cadherin genes are mapped on the long arm of chromosome 18 adjacent to each other. The transcription for the Desmocollin and Desmoglein genes are in opposite directions [10-12]. DSC3 is a homophilic adhesion molecule and so cells expressing DSC3 will adhere to each other at the site of DSC3 expression. It was first cloned from human Bladder cancer cell line [13,14]. Unique distribution and characteristic of DSC3 makes it a unique target for developing novel therapies.

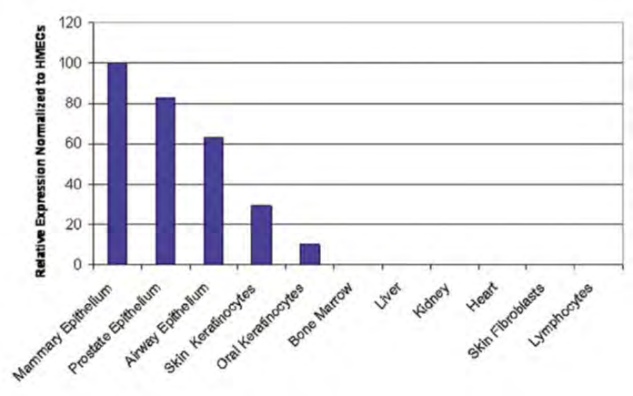

Figure 1: DSC3 expression relative to human mammary epithelium cells
(Source: Breast Cancer Res. 2005;7(5): R669-80.)

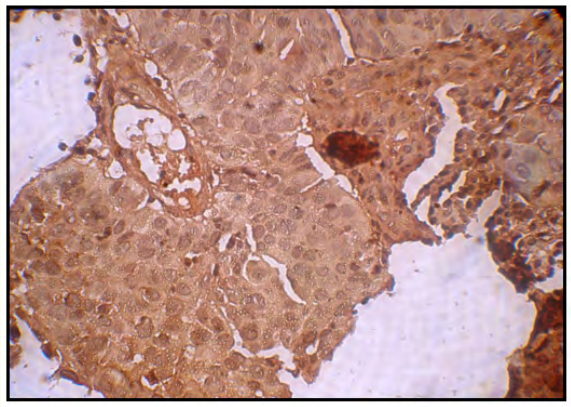

Figure 2: DSC3expression in Squamous NSCLC (immunohistochemistry).

p53 is a tumour suppressor protein. It acts as a checkpoint in the cell cycle and prevents or initiates programmed cell death [11]. DSC3 is a p53-responsive gene [1,16,17]. Endogenous expression of p53 is associated with DSC3 expression. Exogenous expression of p53 increases expression of desmocollin-3. DNA damaging agents Cisplatin, Paclitaxel, Doxorubicin, Gemcitabine etc are known to increase p53 expression and conversion of DSC3 negative to DSC3 positive [15-17].

Point mutation in the p63-binding site suggesting direct control of p63 for DSC3 enhancement. Antibodies against p63 binds to DSC3 genomic regions along with Desmoplakin and Desmogleon-1 [18].

Lung cancer

DSC3 is not seen in normal lung tissue [19]. Basal layers of columnar epithelia (e.g. bronchi and trachea) occasionally express DSC3. Immunohistochemistry may have weak staining in bronchial epithelial cells, at the basolateral borders of suprabasal cells and at the apical side of ciliated cells. In NSCLC cohort, DSC3 expression is seen in around 30% of cases [20]. It is expressed in squamous variety of NSCLC while not expressed in Adenocarcinoma (Figure 2). DSC3 gene is downregulated in adenocarcinoma [21].

Even in lung cancer expression of DSC3 is seen at basal layers of tumor. It is closely associated with p63 expression which is an another marker used for differentiation of Squamous NSCLC from other varieties [22-24]. However, it is more specific for squamous NSCLC as p63 is also expressed in Adenocarcinoma. Presence of DSC3 and TTF-1 are mutually exclusive and are very specific for Squamous NSCLC and Adenocarcinoma respectively [22,23]. The mutual exclusiveness is also seen in large cell carcinoma. Both are expressed in some adenosquamous type of NSCLC [22-24,26,27]. Maximum sensitivity is seen in highly differentiated tumors and is lowest for poorly differentiated Squamous NSCLC. Sensitivity of DSC3 for squamous NSCLC is 93.2% in large cohort of 426 but drops to 59% in poorly differentiated squarmous NSCLC (Table 1). DSC3 expression in NSCLC is also not related to stage or histologic grade [20]. of a disease.

Use of Immunohistochemistry for detection of DSC3 aids in histological diagnosis with a minimal tissues as obtained by biopsy and also helps in management of large cell or other varieties (not otherwise specified) of NSCLC by classifying them in Squamous or non squamous variety [19,24]. DSC3 is not expressed in neuroendocrine NSCLC [25]. Analysis of smaller clinical trials suggest that DSC3 expressing tumors are likely to have better survival compared to DSC3 negative tumors and may serve as a potential prognostic marker [1,20].

S. No	Reference	Squamous		Adenocarcinoma		Large cell carcinoma	
		DSC-3+ve /Total	DSC-3 +ve %	DSC-3 +ve /Total	DSC-3 +ve %	DSC-3 +ve /Total	DSC-3 +ve %
1	Warth., *et al.* (7)	425/456	93.2%	5/530	1.0%	1/60	1.2%
2	Kim., *et al.* (8)	156/171	91%	0/110	0%	-	-
3	Tsuta., *et al.* (9)	109/150	72.70%	0/157	0%	-	-
4	Righi., *et al.* (10)	13/16	86.70%	0/29	0%	12-Feb	16.70%
5	Monica., *et al.* (11)	24/24	100%	1/40	2.50%	28/69	40.60%
	Total	727/817	88.70%	6/866	0.69%	31/141	11.70%

Table 1: Sensitivity of DSC3 for squamous NSCLC.

Sr. No	Parameters	Control	Cisplatin 2.5 µg	Paclitaxel 2.5 µg	Doxorubicin 2.5µM	Gemcitabine 1.0 µg
1	A549 (Human alveolar Adenocarcinoma)	Negative	Positive	Positive	Positive	Positive
2	Panc-1(Human Pancreatic)	Negative	Positive	Positive	ND*	Positive
3	AsPC-1(Human Pancreatic)	Negative	Positive	Positive	Positive	Positive
4	Mia-Pa-Ca2 (Human Pancreatic)	Negative	Positive	Negative	Positive	Positive
*Not done						

Table 2: Effect of chemotherapeutic agents on DSC3 status of DSC3 negative cell lines.

Melanoma

DSC3 is expressed in melanoma [28,29]. Its expression decreases with increased thickness and progression to metastatic melanoma [28-30]. There is a significant drop in DSC3 expression in metastatic melanoma compared to primary cutaneous melanoma.

Colorectal cancer

Methylation of DSC3 DNA [16]. was detected in 41 out of 99 primary colorectal tumours (41.4%). Methylation of DSC3 is found to be associated with poor prognosis. Methylation of DSC3 DNA was found in 23 out of 39 (59%) patients who had a survival time < 5 years, compared to 30% of the patients (18 out of 60) with survival time >5 years, reaching statistical significance (P = 0.004). The effect of methylation on clinical outcome by Kaplan–Meier analysis, also revealed tumours with methylated DSC3 DNA were significantly correlated to a worse clinical outcome than unmethylated tumours (P = 0.002).

Breast cancer

DSC3 is expressed in normal breast but is down-regulated in breast cancer cell lines and primary breast tumors at protein as well as gene level [3,33]. DSC3 gene expression is silenced or markedly reduced (less than 10% of the expression seen in human mammary epithelial cells) in majority of breast cancer cell lines, as well as, in 18 of 24 (75%) of the invasive ductal carcinoma , 5 of 7 (71%) invasive lobular carcinomas [3]. DSC3 protein is not expressed in breast tumor cells with undetectable DSC3 mRNA levels. Protein expression was analyzed by western blot analysis.

Bladder cancer

DSC3 was first cloned from a bladder cancer [13]. Like many epithelial cancers loss of desmocollin-2 expression is known in transitional cell carcinoma [34]. It was found in none of the 85 samples of transitional cell carcinoma evaluated [34]. DSC3 gene is described to be over expressed in bladder cancer and it is suggested to be useful as a diagnostic or therapeutic purpose [35]. We have seen DSC3 to be present in around 90% (27 of 30) of newly diagnosed non muscle invasive bladder cancer.

Other tumors

Meningioma

DSC3 expression is described in 20 0f 32 meningiomas with strong overall positive in two cases and few cell clusters in rest [36].

Chondrosarcoma

DSC3 gene expression is detected in 4 of the 5 chondrosarcoma cell lines examined, with a 2- to a 12-fold increase in expression [37].

Pediatric acute lymphoblastic leukemia

DSC3 gene is described to be over expressed in all TEL-AML1 subtype of paediatric acute lymphoblastic leukemia (ALL) and none in other types of ALL thus differentiating TEL-AML1 from other subtypes of pediatric acute lymphoblastic leukemia [38].

Skin tumors

- Loss of DSC3 is associated with increase in K-Ras induced skin tumors [39].
- A431 cell line from epidermoid cancer is described to be expressing DSC3 protein [40].

Oral squamous cell carcinoma

Oral mucosa normally expresses DSC3. However development of oral Squamous cell carcinoma is associated with reduction or absence of DSC3 expression. This was seen in 30 out of 48 oral Squamous cell carcinoma. This reduction/absence of DSC3 expression was associated with higher histological grade (moderately or poorly differentiated) [41].

Effect of demethylating agents

One of the reasons for non-expression of DSC3 is epigenetic silencing. Methylation of DSC3 DNA is one of the major factors accounting for epigenetic silencing [3,4,27,34]. Methylation of DSC3 DNA is the main reason for absence of DSC3 in breast cancer [3]. This is also seen as one of the major mechanism for absence of DSC3 expression in colorectal cancer and adenocarcinoma of lung [3,31]. Demethylating agent, 5-aza-2'-deoxycytidine (DAC), induces expression of DSC3 in select cell lines not expressing DSC3 [3]. Methylation status of DSC3 is associated with poor prognosis compared to nonmethylated DSC3 [3,34].

Effect of EGFR inhibitor

Epidermal growth factor receptor (EGFR) has inverse relationship with DSC3 [37]. Activation or increase in EGFR leads to decrease in expression of DSC3 while EGFR inhibitors increase expression of DSC3. EGFR inhibitors also converts DSC3 negative cell lines to DSC3 positive. This has been demonstrated for lung cancer cell lines [35].

Desmocollin-3 and cancer therapy outcomes

DSC3 is an adhesion molecule. Cells expressing DSC3 remain adherent to each other. This prevents mobility and there by spread of cancer. This phenomenon has been demonstrated to be advantageous in form of improved outcomes in patients expressing DSC3 compared to patients who do not express DSC3. The advantage in outcome has been demonstrated in non small lung cancer, colorectal cancer etc. [16].

Cadi-05 (Currently marketed as Mycidac-C for NSCLC in India)

Cadi-05 is a potent TLR-2 agonist which enhances pure Th1 response by activation of CD4+ and CD8+ T cells, NK cells and also macrophages [43]. It also induces expression of DSC3 on immune cells and directs immune response towards DSC3. In *in vitro* - ex vivo experiments, it preferentially kills DSC3 expressing cancer cells. In animal models it is found to increase tumor infiltrating CD4+ and CD8+T cells, NK and NKT cells, macrophages and dendritic cells which are activated (Figure 4). It decreases Treg cells in the tumor mass. It also improves efficacy of chemotherapy when both are used to gather [45,46].

Cadi-05 administration results in decrease in Treg cells in draining lymph node as well as tumor mass. Cadi-05 treatment of tumor bearing mice lowers IL-6 but increases IL12p70 and IFNγ in sera. Also, increase in CD8+ T cell mediated lysis of specific tumor targets. This efficacy is reduced in Ifn gamma-/- mice. This effects are also not observed in NOD-SCID mice [44].

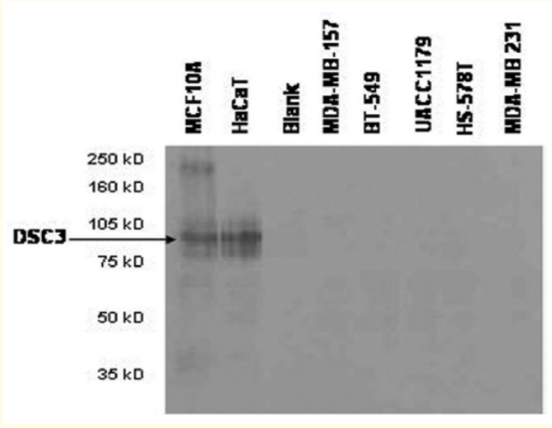

Figure 3: Western blot analysis (Source: Breast Cancer Res. 2005;7(5):R669-80.).

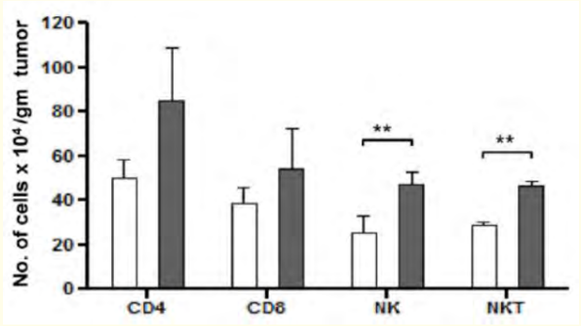

Figure 4: Effect of Cadi-05 on CD4+ and CD8+T cells, NK and NKT cells.
(Source: PLoS One. 2011;6(9): e25424.).

Cadi-05 is heat killed suspension of Mycobacterium W (MW), a non-pathogenic, cultivable atypical mycobacterium. Each dose of 0.1 ml Cadi-05 contains Mycobacterium W (Heat killed) 0.5 x 10^9 cells in normal saline.

Cadi-05 and human clinical studies

The following text highlights ongoing and conducted clinical trials for Cadi-05.

Cadi-05 in NSCLC* [47,48]

In a randomized scheme, 221 patients with newly diagnosed, advanced (stage III and / or IV), treatment naïve NSCLC, as confirmed by histopathology or cytology, were assigned to cisplatin and paclitaxel (control arm) or cisplatin and paclitaxel plus Cadi-05 (test arm) (NCT00680940).

Patients received 3-hour infusion of 175 mg/m2 of paclitaxel and 1-hour infusion of 100 mg/m2 of cisplatin on the first day of a cycle (21 days) for a total of 4 cycles. Cadi-05 (0.2ml as 0.1ml over each deltoid) was administered intradermally, a week before initiating chemotherapy. Subsequently 0.1ml of Cadi-05 was administered on day 8 and 15 of each cycle till 4 cycle and every month for a total of 12 months or till progression of disease/death, whichever was earlier.

After baseline evaluation, tumor status was evaluated at end of 2 cycles and 4 cycles using CT scan and tumor response was performed by an independent radiologist using RECIST criteria. Tumor evaluation was repeated every three months till disease progression or death for one year.

Cadi-05 improved median overall survival in treatment arm by 66 days (HR = 0.64; 95% CI: 0.41-0.98; p = 0.0438) in those who completed four cycles of chemotherapy (Figure 5).This was also associated with improved survival rate by 17.48% at the end of one year (33.89% (20 of 59) vs 16.41%(11 of 67)) in the test arm.

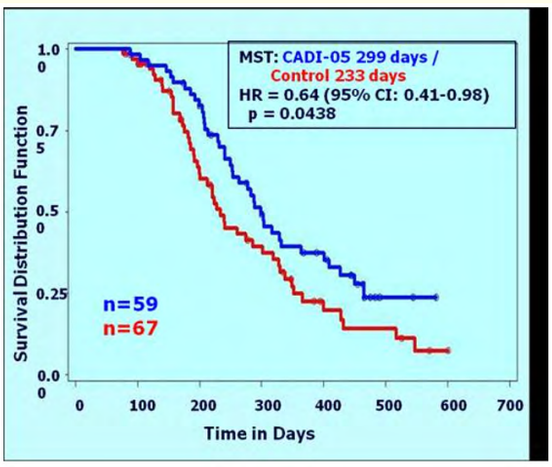

Figure 5: Overall survival in treatment by 66 days.

Improvement in response rate at the end of four cycles of chemotherapy was found to be 10% (37% to 47%) with complete response in 3 patients in the test arm and none in the control arm.

The subgroup analysis reveals maximum treatment benefit in Squamous cell carcinoma which are known to express DSC3 (Figure 6). The survival benefit for those who completed four cycles is 110 days (HR = 0.40; 95% CI: 0.17-0.96; p = 0.041). In ITT analysis as well as from those who completed four cycle of chemotherapy. The survival benefit from ITT is 47 days (HR = 0.54; 95% CI: 0.31-0.94; p = 0.0312).

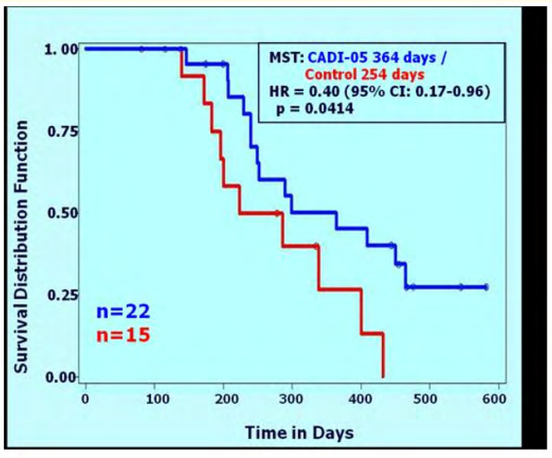

Figure 6: Survival benefit in treatment for those who completed 4 cycles in 110 days.

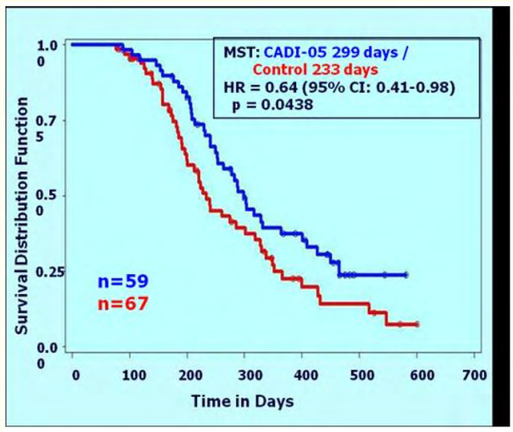

Figure 7: ITT Analysis: Squamous cell carcinoma.

Figure 9: Minimal residual tumor seen following 3 injections of Cadi-05 & 20 GY of radiation.

Cadi-05 in bladder cancer

BCG resistant/recurrent non muscle invasive bladder cancer

In a pilot study (NCT00694798) 22 patients with BCG resistant/recurrent non muscle invasive bladder cancer were treated with intradermal cadi-05 following transurethral resection. 11 of 22 (50%) subjects were found to be disease free at the end of six months with seven remaining disease free till 15 months (end of study period) [48].

Cadi-05 was administered every two weeks for six administration followed by every 4 weeks for six administrations. It was subsequently give every 8 week. It was administered as 0.2 ml at first visit and 0.1 ml subsequently. All administrations are intradermal.

Advanced bladder cancer

In a preliminary study of advanced bladder cancer, cadi-05 administration along with radiotherapy resulted in complete response which was maintained for at least two years (Figure 8-10). Five patients had T3a disease and one had T4b disease. All the patients disease free and remained disease free till 2 years. All patients received Cadi-05 every two weeks for six months [49].

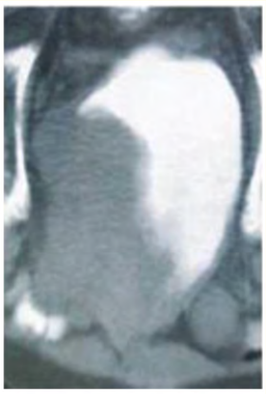

Figure 8: Muscle Invasive bladder cancer extending to abdominal wall. Intravesical tumor measures 8.5cm * 7.6 cm.

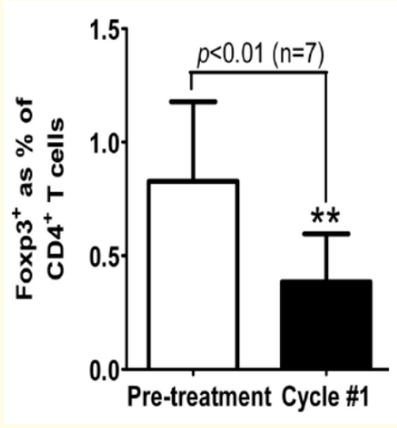

Figure 10: Effect of Cadi-05 on circulating T-cell.

Cadi-05 in melanoma

In a pilot study (NCT00675727) in metastatic melanoma (stage IV), Cadi-05 as a monotherapy, when administered intradermally provides anti-tumor response. Cadi-05 administration resulted in reduction of circulating serum Treg cells (immunosuppressive cells) (Figure 10). Regression of lung lesions was seen in both patients with lung metastasis (Figure 11).

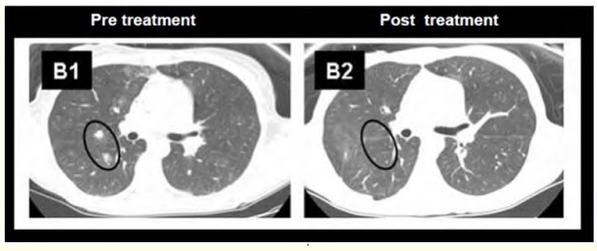

Figure 11: Improvement in survival following Cadi-05.

The improvement in survival following Cadi-05 was found to be better in those who received prior high dose Il=2 compared to those who did not received it (20.7 months 95% CI (10.225, 31.082) versus 5.6 months 95% CI (3.90, 7.38), p = 0.03) [51].

Two of the patients could undergo surgery for residual disease following Cadi-05 treatment and achieved a status of "no evidence of disease" which was maintained till last follow-up.

Cadi-05 in Head and neck cancer

Cadi-05 has not been found to improve outcome of therapy in head and neck cancer (DSC3negative) as a monotherapy as well as when combined with chemoradiotherapy [52-54].

Cadi-05 in Prostate cancer

Cadi-05 was also evaluated in metastatic hormone refractory prostate cancer along with Docetaxel. The trial (NCT00525408) was discontinued after interim analysis as DSMB felt that it is not likely to provide any benefit. Hormone refractory prostate cancer is DSC3 negative. Mw appears to improve steroid resistant optic neuritis.

Cadi-05 use as intravenous infusion in Optic neuritis

Investigated the safety and outcomes of off label immunomodulator Mycobacterium w. (Mw), a TLR 9 antagonist in steroid-resistant idiopathic unilateral optic neuritis. Mw 5 ml in 500 ml normal saline, 30 days after the last of dose of steroids had been administered. The dose was repeated at 3 months. Mw has been shown to be extremely safe and showed no adverse events, a result reaffirmed by this study [56].

Adverse event and prevention

Severe injection site reaction, large ulcers and abscesses are most commonly caused by faulty injection technique where part or the entire dose is administered too deeply (subcutaneous instead of intradermally).

Keloid formation at injection site is an uncommon and largely avoidable, complication of Mycidac-C. Most experience has been gained in the use of the upper arm and it is known that risk of keloid formation is increased manifold when the injection is given at a site higher than the insertion of the deltoid muscle near the middle of the upper arm.

Precautions

- Local site reactions are likely to occur within two to six weeks at the injection site. It starts as a small papule which increases in size over the next few weeks with scaling, crusting and occasional bruising. They are usually minor and self limiting and sometimes lead to a small self healing ulcer.
- Inadvertent subcutaneous or intramuscular administration of Mw leads to severe injection site reactions in the form of large ulcers and abscesses.

Management of the local site reactions

- If lesion occurs, it is not necessary to protect the site from becoming wet during washing and bathing, but in case of oozing, a temporary dry dressing may be used until a scab forms.
- It is essential that the site is exposed to air. If it is absolutely essential to cover it then an impervious dressing may be applied but only for a short period, as it may delay healing and cause a larger scar.

Discussion

DSC1 may be a marker for tumour differentiation, DSC3 has a potential diagnostic value in subclassification of non-small cell lung carcinoma into SCC and ADC, and furthermore, DSC1 and DSC3 may be prognostic markers for lung cancer. Desmocolin-3 is important target for other cancer like bladder cancer, melanoma, Head and neck cancer and colorectal cancer. Currently no treatment option available for DSC3 expressed tumor. Mycidac-C (mycobacterium-w) is a world's first active immune therapeutic agent targeting DSC3. It is a pure potent Th1 response enhancer. Mycidac-C is approved by DCGI for NSCLC patients along with chemotherapy. Approved route of admiration is intradermal. Inadvertent subcutaneous or intramuscular administration of Mw leads to severe injection site reactions in the form of large ulcers and abscesses. For optic neuritis intravenous Mw 5 ml in 500 ml normal saline, 30 days after the last of dose of steroids had been administered and found to be safe without any side effect i.v Mw should be preferred route of administration for cancer chemoimmunotherapy. Desmosomal proteins have also been considered as prognostic markers in various cancer types. For example, downregulation of desmoplakin expression provides prognostic information in human oropharyngeal cancer. Decreased DSG3 expression was associated with poor

prognosis in lung cancer. methylation of DSC3 DNA is a prognostic marker in human colorectal cancer. In this study, we found that reduced expression of DSC1 and DSC3 was associated with an unfavourable prognosis in lung cancer. Like DSG3, DSC1 and 3 seem to be a potential prognostic marker in lung cancer. In summary, our data showed that DSC1 may be a marker for tumour differentiation, DSC3 protein expression may have diagnostic value for subclassification of NSCLC into SCC and ADC; moreover, patients with reduced DSC1 and DSC3 expression had poor prognosis. Further studies are needed to explore the functional role of DSCs-3 in lung carcinogenesis.

Conclusion

Cadi-05 (Mycidac-C) an world's first active immunotherapy directed against DSC3 offers a new treatment option in management of DSC3 expressing tumors as a monotherapy when tumor burden is small as in bladder cancer following transurethral resection and along with chemotherapy when tumor burden is more as in advanced non- small lung cancer with squamous histology.

- Cadi-05 in management of advanced non small lung cancer is accepted for approval by Indian regulators.
- Cadi-05 has also received orphan drug designation from office of Orphan drug designation of US FDA for DSC3 expressing non- small cell lung cancer.

Acknowledgement

This is a collaborative work of a Cadi-05 team which includes scientists within and outside Cadila Pharmaceuticals Limited, Ahmedabad.

Financial support and sponsorship

Nil.

Conflicts of Interest

Yes, All author are employee of Cadila Pharmaceuticals Ltd, Cadila sold the product Mycidac-C (Cadi-05) which targets Desmocolin-3 positive cancer.

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