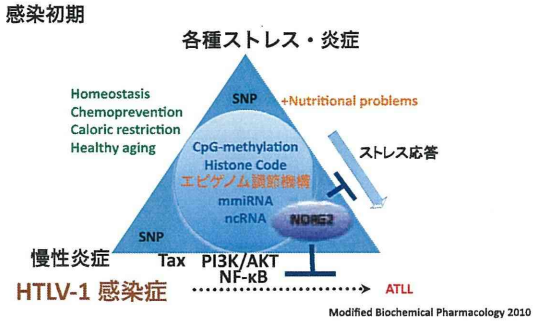
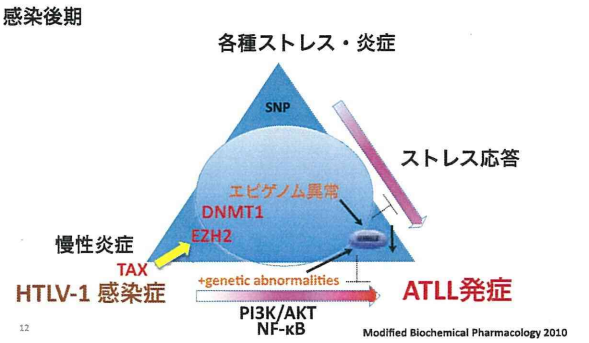
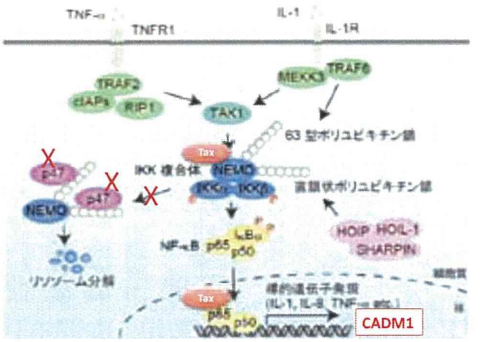
## NDRG2は慢性炎症及び各種ストレス応答を負に制御する




## TAX/EZH2活性化によるNDRG2発現低下はATLL発症に繋がる

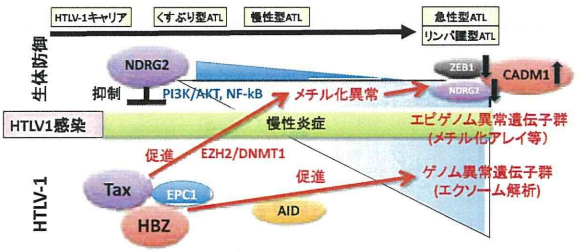



## ATL新規マーカーCADM1の高発現はTAX及びp47低発現によるNF-kB活性化に依存している




東大医科研・井上研究室を改変

## HTLV-1感染からATL発症モデル




HBZは慢性炎症を促進、AIDA発現亢進、ゲノム異常を促進する。一方TAXはEZH2/DMT2を活性化し、生体防御に関わるNDRG2遺伝子等の転写を抑制し、さらなるエピゲノム異常の蓄積に関わりZEB1発現低下、TSLC1高発現に関わりATL発症に繋がる可能性を示唆

厚生労働科学研究費補助金
第3次対がん総合戦略研究事業

## ATLの腫瘍化並びに急性転化、病型変化に関連する遺伝子群の探索と病態への関与の研究

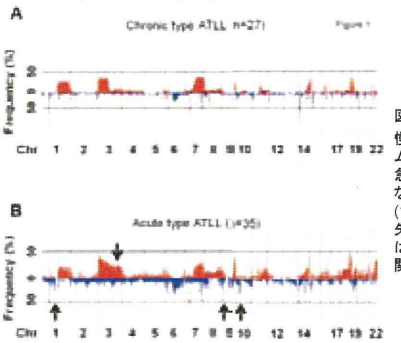
代表者名　:愛知県がんセンター研究所　瀬戸　加大

役割分担:

瀬戸　加大　研究の総括、ゲノム異常解析、遺伝子発現解析、遺伝子機能解析
都築　忍　遺伝子機能解析(In vitro解析およびマウスモデルの作成)
大島　孝一　病理組織診断と残余検体の凍結保存、FISH, in situ検索
宇都宮　與　臨床検体の収集と臨床病態との関連の研究
今泉　芳孝　臨床検体の収集と臨床病態との関連の研究

### 1. 慢性型ATL(27症例)、35症例のゲノム異常解析:

一急性型ATLに特徴的なゲノム異常領域が明らかになった。



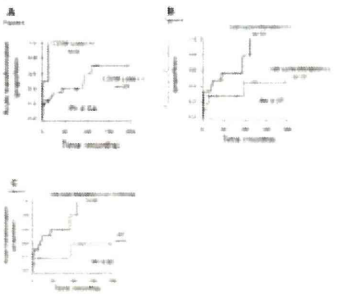

図脚注:
慢性型ATLと急性型ATLのゲノム以上様式はよく似ているが、急性型では慢性型には見られないゲノム異常領域があった(1p13欠失, 3q増幅、9p21.3欠失, 10p11.2欠失:矢印)。これらは、急性型ATLの病因、病態に関与すると考えられた。

### 2. 急性転化に関与する遺伝子の一部が判明:

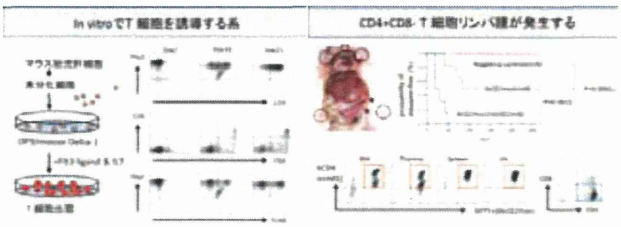
一ゲノム異常と発現遺伝子差違の相関を検討し、これらの4領域のうち3領域の責任遺伝子CDKN2A(9p21.3)、CD58(1p13)、CCDC7(10p11.2)、ITGB1(10p11.2) を明らかにした。

### 3. 慢性型の急性転化に関わる予後予測マーカーの確立:

一慢性型ATLの急性転化に関わるバイオマーカー(予後不良マーカー)として、細胞周期制御遺伝子群の異常とCD58遺伝子の欠失を見いだした。



**4. マウス正常T細胞培養系を用いたマウス実験モデルの作成:**
**—OP9/DL1フィーダー、Flt ligand、転座関連遺伝子を用いて CD4+CD8−のT細胞リンパ腫の作成に成功した。**




HTLV-1ウイルスを起因として発症する成人T細胞性白血病リンパ腫（ATL）は感染者のうち2-5%が発症する。これはウイルスに加え、ウイルス感染細胞にゲノム異常がさらに複数加わって腫瘍化することを示唆する。本研究の目的は、ATL疾患単位を形成する特徴的なゲノム異常領域から責任遺伝子を見いだし、腫瘍化や急性転化、病型変化などへの関与を機能的に解明し、病型変化の早期発見のマーカーを確立することである。ATLの臨床病型の中で、慢性型ATLは均一な病態を示し緩徐進行性な病型と考えられていたが、うち半数例が急性型へ移行し、死亡している。このことから慢性型ATLに着目し、急性型ATLと合わせて分子病態の解析を実施した。

27例の慢性型ATLならびに35例の急性型ATL（うち1例は慢性型から急性型への連続サンプル）を対象とし、オリゴアレイCGHでそのゲノム異常解析を実施した。両病型のゲノム異常様式は似通っていたが、幾つか急性型ATLでのみ高頻度に認めるゲノム異常部位が存在していた。このうち9p21.3部のゲノム欠失は慢性型ATLと比べて急性型ATLで特に特徴的であったため同部に着目した。その欠失部に存在する遺伝子のうち、CDKN2AのmRNA発現値のみが9p21.3欠失に伴い低下していた。1例得られた連続サンプルでの評価でも、急性転化期につれて9p21.3にゲノム欠失が生じ、CDKN2Aの発現値が著減していた。このためCDKN2Aは急性転化に関与する責任遺伝子の一つと考え、機能解析をATL 細胞株を用いて実施した。CDKN2A(INK4aとARF)の導入によりATL細胞株の増殖抑制を認め、ATLの病態生理においてがん抑制遺伝子として機能していることを確認した。CDKN2Aは細胞周期の負の調節因子として働くことが知られている。このことから、細胞周期の脱制御が急性転化機構にとって重要であると考えられる。臨床像として、慢性型ATLの中で細胞周期関連遺伝子部にゲノム異常を有する群は有しない群に比べて有意に予後不良であり、また累積急性転化発症率も高い傾向を認めた。このことは、CDKN2Aを始めとする細胞周期関連遺伝子が慢性型ATLの急性転化に関与していることを示しており、またこれら遺伝子は新規急性転化予測マーカーとなりうる可能性がある。

厚生労働省第3次対がん HTLV-1関連疾患研究領域 合同発表会
（平成 26年 2月 8 日）

## 細胞接着・運動性経路を標的とした ATL 細胞の浸潤、増殖抑制医薬品開発のための基礎研究（23120701）

研究代表者：村上 善則「分子腫瘍学的解析」
（東京大学医科学研究所 人癌病因遺伝子分野）

分担研究者：内丸 薫「血液内科学的研究」
（東京大学医科学研究所附属病院 血液内科）

分担研究者：後藤 明輝「病理学的研究」
（秋田大学大学院医学研究科 病理学）

厚生労働省第3次対がん HTLV-1関連疾患研究領域 合同発表会
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## 細胞接着・運動性経路を標的とした ATL 細胞の浸潤、増殖抑制医薬品開発のための基礎研究（23120701）

1) ATL特異的なCADM1分子経路の解析と低分子阻害剤の検索

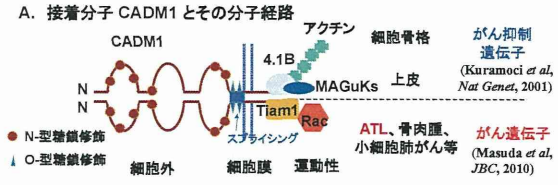
2) ATL 細胞のCADM1糖鎖修飾の解析と高親和性抗体作成の試み

3) CADM1 の発現を抑制する siRNA, miRNAの同定とレンチウイルスベクターによる発現

4) CADM1 を表面マーカーとして用いた悪性 ATL 細胞の診断（分担研究者：内丸薫博士）

### 免疫グロブリンスーパーファミリー細胞接着分子CADM1はATLに特徴的に発現する浸潤・転移抑制の標的分子である

A. 接着分子 CADM1 とその分子経路



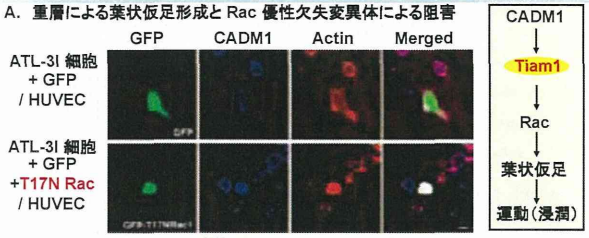

B. CADM1 を標的としたがんの浸潤・転移の抑制

CADM1 はATLで特異的に高発現し、ATL 細胞の浸潤能を高めるがん遺伝子として作用する。(Sasaki , Moroshita et al, Blood, 2005; Masuda et al, JBC, 2010)

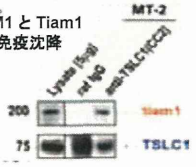
がん遺伝子 → ATL の発症前診断、質的診断 → 診断薬の開発
がん遺伝子 → ATL の浸潤・転移抑制医薬品の標的分子 → 治療薬の開発

### CADM1/TSLC1 は Tiam1、Rac を介して ATL 細胞の葉状仮足形成を導き、細胞浸潤を促進する

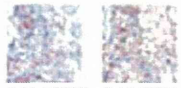
A. 重層による葉状仮足形成と Rac 優性欠失変異体による阻害




B. CADM1 と Tiam1 との免疫沈降




C. ATL 患者リンパ節での発現

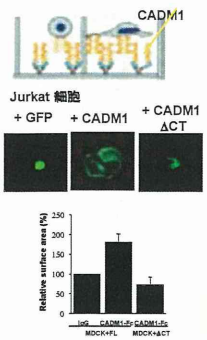



過剰発現: 9 / 9 (100%)　3 / 9 (33%)

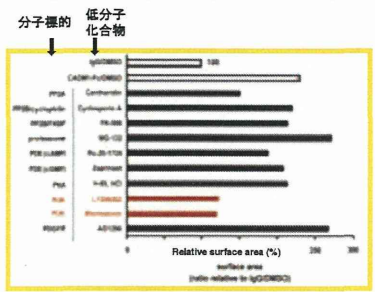
(Masuda et al, JBC, 2010)

### 課題 1. CADM1を介する細胞伸長を抑制する化合物の同定

A. 固相化CADM1 細胞伸長アッセイ



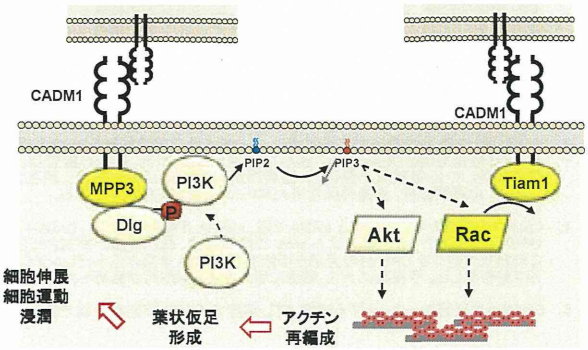

B. 92 既知低分子化合物の伸長アッセイによる検索




CADM1 を介する細胞伸長反応は、PI3K 阻害剤により、阻害される。

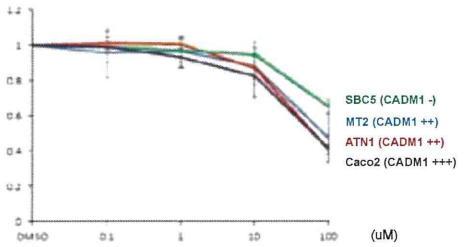
(Murakami S et al, PLoS ONE, in press)

### 課題 1. CADM1 を介する細胞伸展、細胞浸潤に関わる分子経路



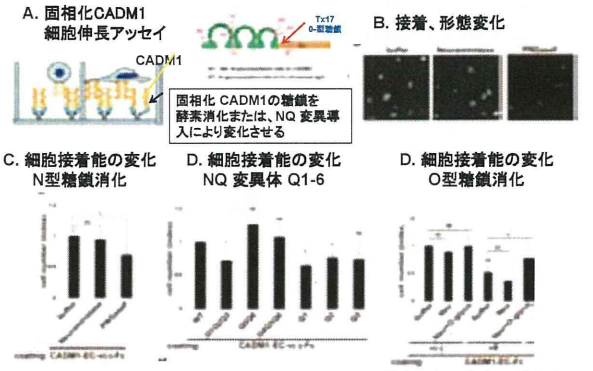

(Murakami S et al, PLoS ONE, in press)

## 課題 1. PI3K 阻害剤 LY294002 による ATL、癌細胞の増殖抑制



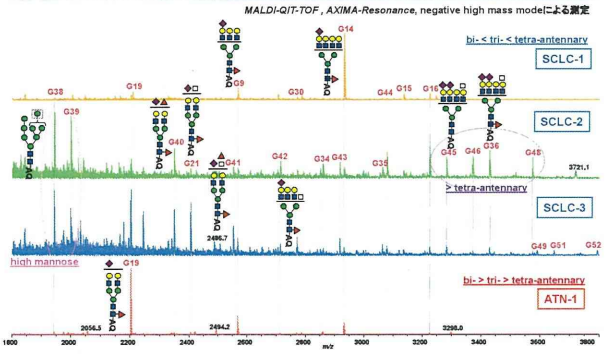

ATL 細胞、癌細胞CADM1を高発現する ATN1, MT2 細胞は、
PI3K 阻害剤 LY294002 に増殖抑制感受性を示す

## 課題 2. CADM1の糖鎖は、がん細胞の細胞接着、伸長能を 修飾する N型糖鎖は促進し、O型糖鎖は抑制する



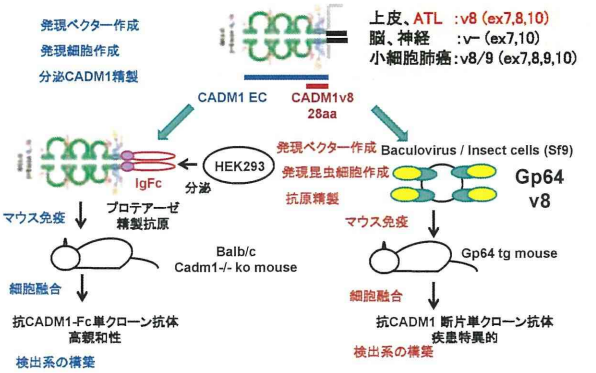

(Sakurai *et al.*, in revision)

## 課題 2. CADM1の*N*型糖鎖プロファイル（島津製作所との共同研究）



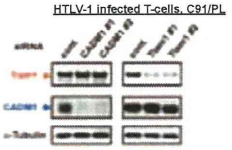

ATL細胞では、上皮細胞と比較して、複合型2分岐構造の
N-型糖鎖修飾が多く認められる

## 課題 2. 抗 CADM1 単クローン抗体作成（東大先端研と共同研究）

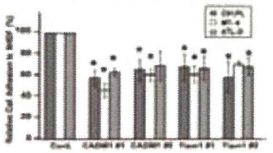



## 課題 3. CADM1, Tiam1 siRNA による ATL悪性形質の増悪

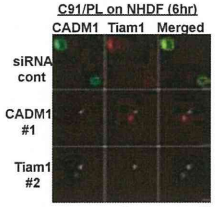
A. RNAi による発現抑制



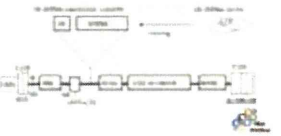

B. CADM1、Tiam1 の発現抑制による ATL
細胞の線維芽細胞 への接着能低下



C. CADM1 、Tiam1 の発現抑制
による葉状仮足形成能の消失

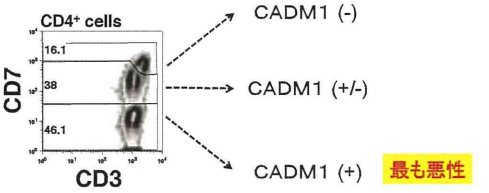



D. CADM1発現レンチウイルスの作成へ



## 課題 4. 悪性 ATL の特異的マーカーとしての CADM1 の確立
（分担研究者内丸薫博士の研究）

-Non-symptomatic carrier
-Smoldering ATL

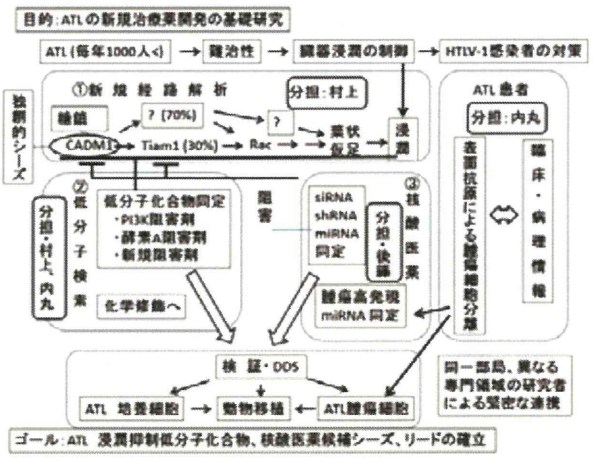



(Kobayashi S, Uchimaru K *et al*, *PLoS ONE*, 2013)

CADM1は悪性 ATL 細胞の特異的診断マーカーとして利用できる

## まとめ

1. ATL で特異的に発現し、細胞浸潤を促進する膜分子 CADM1を標的として、ATL の新規診断、治療薬を開発する目的で、細胞の形態の伸展を指標として、ATL細胞における CADM1経路阻害剤のスクリーニング系を構築した。
2. この系を用いて PI3K阻害剤が CADM1による細胞伸展を阻害することを見出し、新たな下流分子経路 CADM1-MPP3-DLG-PI3K-AKT, RAC1 を同定した。CADM1を高発現する ATL 細胞は、PI3K 阻害剤 LY294002 に対して、一定の感受性を示した。
3. CADM1の N型、O型糖鎖構造を解析し、複合鎖2分岐構造を示す N-型糖鎖が、ATL細胞で比較的多く認められることを見出した。また、細胞の接着能、伸展能に対して N-型糖鎖が促進、O-型糖鎖が抑制に働くことを示した。現在、CADM1 の高親和性、疾患特異性モノクローナル抗体を作成中である。
4. CADM1の発現を強く抑制する siRNA 2種、miRNA 2種を同定した。CADM1 siRNA を発現した ATL 細胞は *in vitro* の細胞増殖、細胞凝集が抑制された。この知見に基づき CADM1の発現を抑制する shRNA を組み込んだレンチウイルスを作成した。予備的に ATL 細胞に導入すると細胞死が認められた。
5. CADM1を表面マーカーとする悪性 ATL 細胞の FACS 診断が可能である。



## 「miRNAを用いたATLがん幹細胞特異的新規治療法の開発」

（H24-3次がん一般-004）

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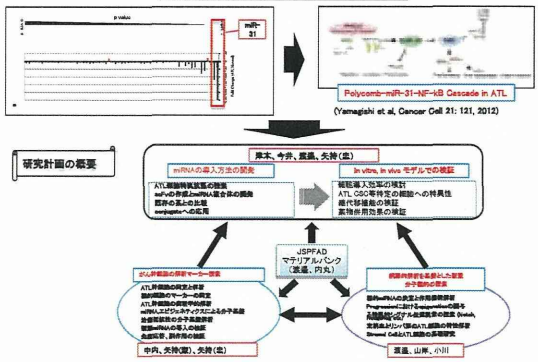
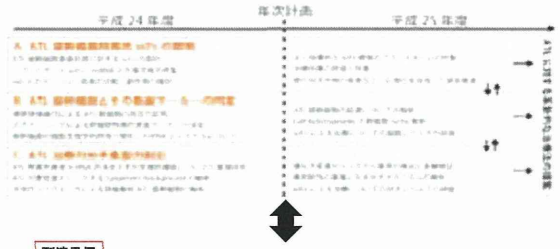
## ATLにおけるmiR-31発現欠損




## 研究計画

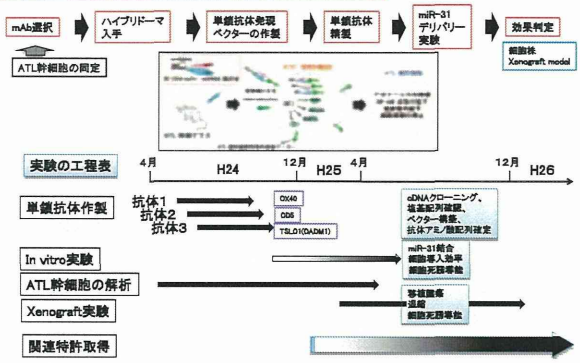


到達目標

1. 単鎖抗体によるmiRNAデリバリーの検証
2. 「ATLがん幹細胞」の分子病態理解の深化と展開
3. ATL細胞の分子異常の理解の深化

→ 新規抗体療法
→ 発症予防と治療の分子標的薬の深化

## 1．単鎖抗体を用いたmiR-31デリバリー法の開発

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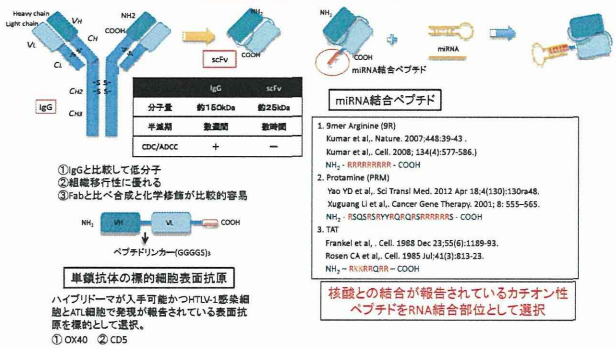
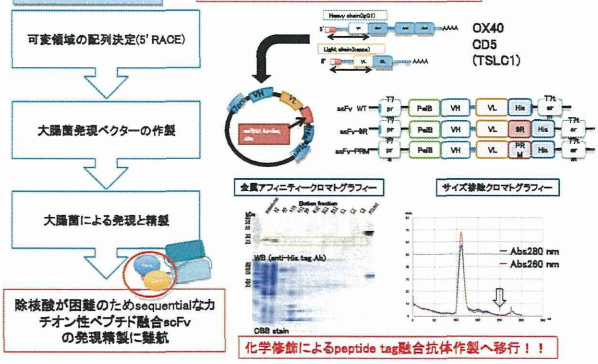
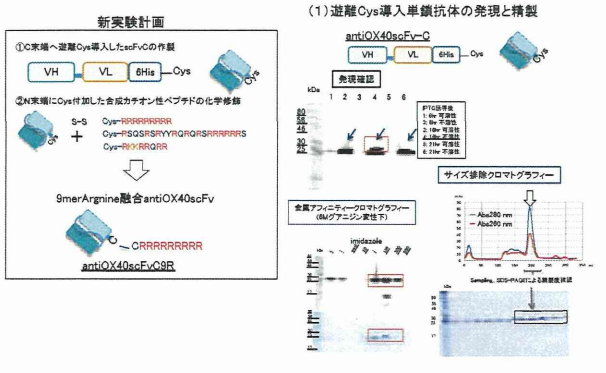


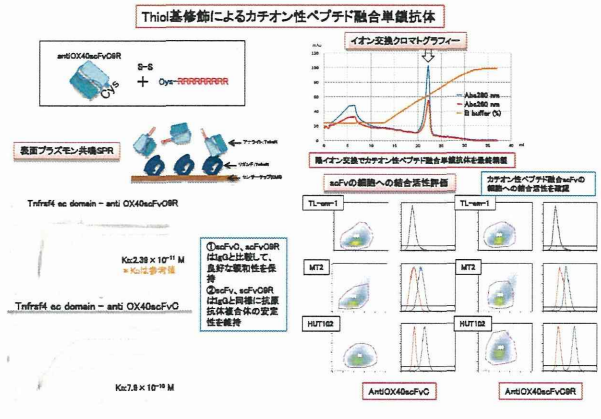

## 進捗状況（1）　カチオン性ペプチド融合scFvの作成



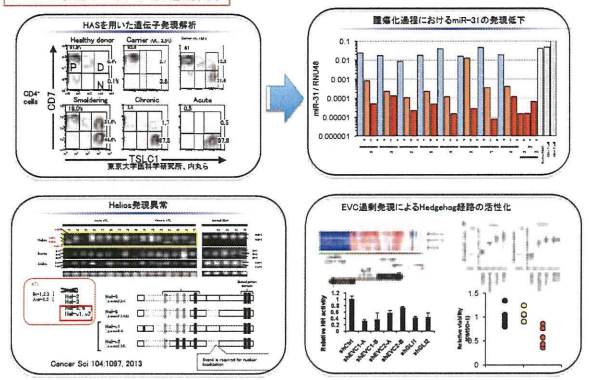

## 進捗状況（2）　化学修飾によるペプチド融合単鎖抗体の作製




## Thiol基修飾によるカチオン性ペプチド融合単鎖抗体

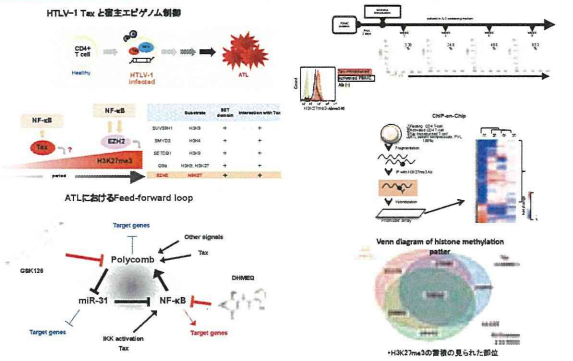


## 2. ATL細胞の分子病態解析



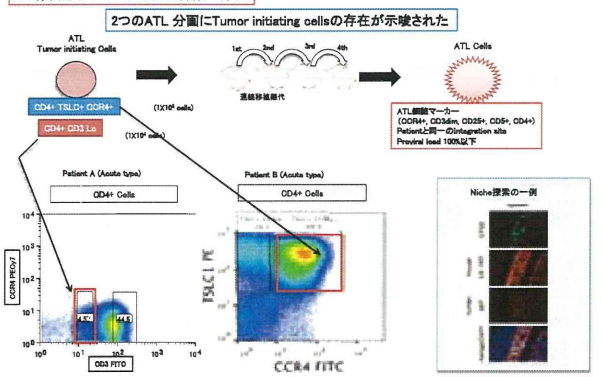

## 2. ATL細胞の分子病態解析

HTLV-1 Tax による不死化とエピジェネティックな影響




## 3. 幹細胞マーカーとNicheの探索の深化

2つのATL 分画にTumor initiating cellsの存在が示唆された




## 要約

1. **単鎖抗体を用いたmiRNA導入法の開発**
   - **カチオン性ペプチド融合の作成⇒困難**
   - **⇒ペプチドの化学修飾による融合scFvの作製**
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2. **ATL細胞の分子病態解析**
   - **不死化HTLV-1感染細胞でのmiR-31発現低下**
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   - **EVC過剰発現によるHedgehog経路の活性化**
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3. **幹細胞マーカーとNicheの探索の深化**
   - **2つのATL 分画にTumor initiating cellsの存在が示唆された**
   - **脾臓におけるNicheの可能性**

# III　研究成果の刊行に関する一覧

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Tsukasaki K, Watanabe T(13人中8番目), Iwatsuki K(13人中10番目), Kamihira S(13人中11番目), Yamaguchi K(13人中12番目), Shimoyama M | Meeting report on the possible proposal of an extranodal primary cutaneous variant in the lymphoma type of adult T-cell leukemia-lymphoma | ***J Dermatol*** | 41 | 26-28 | 2014 |
| Iwatsuki K, Hamada T, Japan Skin Cancer Society-Lymphoma Study Group | Current therapy of choice for cutaneous lymphomas: complementary to the JDA/JSCS guidelines | ***J Dermatol*** | 41 | 43-49 | 2014 |
| Ishihara K, Inokuchi N, Kamihira S(8人中8番目) | Relevance of molecular tests for HTLV-1 infection as confirmatory tests after the first sero-screening | ***J Immunoassay Immunochem*** | 35(1) | 74-82 | 2014 |
| Nishimura J, Yamamoto M, Kanakura Y(23人中23番目) | Genetic Variants in C5 and Poor Response to Eculizumab in PNH | ***N Engl J Med*** | 370(7) | 632-639 | 2014 |
| Miyake A, Fujita M, Adachi A(13人中12番目), Miyazaki Y | Poly-proline motif in HIV-2 Vpx is critical for its efficient translation | ***J Gen Virol*** | 95 | 179-189 | 2014 |
| Nomaguchi M, Yokoyama M, Kono K, Nakayama EE, Adachi, A(17人中17番目) | Generation of rhesus macaque-tropic HIV-1 clones that are resistant to major anti-HIV-1 restriction factors | ***J Virol*** | 87(21) | 11447-11461 | 2013 |
| Sugiyama D, Katayama I, Ezoe S, Kanakura Y(13人中8番目), Sakaguchi S | Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking antitumor immune responses in humans | ***Proc Natl Acad Sci U S A*** | 110 (44) | 17945-17950 | 2013 |
| Umekita K, Umeki K, Miyauchi S, Ueno S, Okayama A(9人中9番目) | Use of anti-tumor necrosis factor biologics in the treatment ofrheumatoid arthritis does not change human T-lymphotropic virus type 1markers: a case series | ***Mod Rheumatol*** | | doi:10.3109/14397595.2013.844389 | 2013 |

| | | | | | |
|---|---|---|---|---|---|
| Asanuma S, Iwanaga M(15人中11番目), Yamaguchi K(15人中12番目), Watanabe T(15人中15番目) | Adult T-cell leukemia cells are characterized by abnormalities of Helios expression that promotes T-cell growth | ***Cancer Sci*** | 104(8) | 1097-1106 | 2013 |
| Mahieux R, Watanabe T | Forefront studies on HTLV-1 oncogenesis | ***Front Microbiol*** | 4:156 | 2pp | 2013 |
| Satoh Y, Yokota T, Sudo T, Kondo M, Kanakura Y(17人中17番目) | The Satb1 protein directs hematopoietic stem cell differentiation toward lymphoid lineages | ***Immunity*** | 38(6) | 1105-1115 | 2013 |
| Tsukasaki K, Tobinai K | Biology and treatment of HTLV-1 associated T-cell lymphomas | ***Best Pract Res Clin Haematol*** | 26 | 3-14 | 2013 |
| Sugaya M, Hamada T, Kawai K, Yonekura K, Iwatsuki K | Guidelines for the management of cutaneous lymphomas (2011): A consensus statement by the Japanese Skin Cancer Society - Lymphoma Study Group | ***J Dermatol*** | 40 | 2-14 | 2013 |
| 齋藤　滋 | HTLV-I母子感染対策 | *産婦人科の実際* | 62 | 543-547 | 2013 |

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MEETING REPORT

# Meeting report on the possible proposal of an extranodal primary cutaneous variant in the lymphoma type of adult T-cell leukemia-lymphoma

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## ABSTRACT

**Based on the advances in research on the clinicopathophysiology of adult T-cell leukemia-lymphoma (ATL), Japanese researchers collected and evaluated cases of smoldering ATL exhibiting primary cutaneous manifestation but showing poor prognosis. Macroscopic findings of skin eruptions were categorized into the patch, plaque, multipapular, nodulotumoral, erythrodermic and purpuric types, as previously reported. Pathological findings were divided into low or high grade based on epidermotropism, tumor cell size and perivascular infiltration. Eight eligible cases were evaluated among 14 collected cases. Macroscopic findings were nodulotumoral in six cases, a subcutaneous tumor in one case and plaque in one case, and the number and size were heterogeneous in each case. Pathological findings of all eight cases were T-cell lymphoma, high-grade type (pleomorphic, medium or large size), with prominent perivascular infiltration and scant epidermotropism. To diagnose such cases as the "lymphoma type of ATL, extranodal primary cutaneous variant", it is essential to examine each case carefully, including cutaneous lesions at onset, lymph nodes and other organ involvement using computed tomography (CT) and/or positron emission tomography/CT, as well as the percentage of abnormal lymphocytes in peripheral blood. Based on the results of an ongoing nationwide survey on ATL, ATL with cutaneous lesions will be analyzed to investigate the incidence and prognosis of the so-called "lymphoma type of ATL, extranodal primary cutaneous variant".**

**Key words:** adult T-cell leukemia/lymphoma, extranodal primary cutaneous variant, lymphoma type adult T-cell leukemia/lymphoma, smoldering adult T-cell leukemia/lymphoma.

## PURPOSE OF THE MEETING

On the basis of the modes of initial presentation and natural history of patients with adult T-cell leukemia/lymphoma (ATL), the four clinical subtypes of acute, lymphoma, chronic and smoldering have been recognized. Diagnostic criteria for the clinical subtypes were proposed[1] and significant prognostic factors were determined in 1991.[2] Since then, patients with ATL were stratified into two groups, aggressive ones consisting of acute, lymphoma and unfavorable chronic types, and indolent ones consisting of favorable chronic and smoldering types, in which the chronic type was further divided into favorable

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and unfavorable according to significant prognostic factors. This stratification was useful for the selection of treatment, in which most patients with aggressive forms were treated with systemic chemotherapy, while those with indolent forms underwent watchful waiting or local therapy only.

In the clinical subtype classification, however, the lymphoma type did not include extranodal variants because of the rarity of such cases at that time. Since then, variants of extranodal lymphoma type such as primary cutaneous ATL and primary gastrointestinal ATL have been reported. The extranodal primary cutaneous variant included in smoldering type made it particularly difficult for physicians to choose the initial treatment.[3–6] Furthermore, the extranodal primary gastrointestinal variant included in the acute type was reported to respond to treatment and be associated with long-term survival. On the contrary, the localized lymphoma type, which was rare in the initial survey in Japan, was reported to consist of approximately 10% of acute and lymphoma types of ATL, and was associated with relatively favorable prognosis after chemotherapy in a recent nationwide survey in Japan.[7]

Based on the advances in research on the clinicopathophysiology of ATL as described above, Japanese researchers, focusing on ATL, joined by the support of a grant (H23-gan rinsho-ippan-022), collected and evaluated cases such as of the localized lymphoma type and extranodal variants originating from several organs to reconsider the subclassification for the appropriate selection of treatment.

This research group, consisting of Japanese hematologists, dermatologists, pathologists, epidemiologists and oncovirologists, aimed at collecting cases as follows: smoldering type with primary cutaneous manifestation resulting in poor prognosis, acute type with the manifestation of an extranodal variant of primary gastrointestinal or nasopharyngeal type, and localized lymphoma type, reviewing clinicopathological findings and proposing the consensus report.

This report summarizes the discussion of the first meeting on this project, focusing on the extranodal primary cutaneous variant.

## ELIGIBILITY CRITERIA OF PATIENTS FOR THE EVALUATION

Eligibility criteria included smoldering ATL with only cutaneous lesions confirmed by histopathology, and with survival after diagnosis of less than 1 year as a rule but less than 3 years being allowed. Each dermatologist/hematologist picked up the cases, and filled out the case report forms with macro-photographs and histological specimens of cutaneous lesions. We categorized the macroscopic findings of skin eruptions into the patch, plaque, multipapular, nodulotumoral, erythrodermic and purpuric types, as previously reported.[6] When multiple types of skin eruption exist in a patient, the most severe type should be described if a consensus on the hierarchy of severity in the types exists: patch and plaque were considered the lowest and second lowest severity, respectively, and nodulotumoral was most severe. There was no consensus on multipapular, erythrodermic and purpuric types, but multipapular and purpuric types were considered intermediate between nodulotumoral and plaque, and should be described separately. Erythrodermic type should still be carefully evaluated. Subcutaneous tumors were specified but included as the nodulotumoral type.

Pathological findings were divided into low or high grade based on epidermotropism, the cell size and perivascular infiltration.[3]

## RESULTS

Fourteen cases were evaluated, but six of them were deemed ineligible because of the period from the onset of cutaneous lesions to the diagnosis of ATL being more than 4 months in five cases and concurrent lymph node lesions at onset not indicating the smoldering but acute type in one case. Case reports were provided by Dr Y. Sawada (University of Occupational and Environmental Health, Fukuoka), Dr Y. Uchida (Kagoshima University, Kagoshima), Dr T. Johno (Kumamoto University, Kumamoto), Dr M. Takenaka (Nagasaki University, Nagasaki), Dr K. Uchimaru (Tokyo University, Tokyo) and Dr K. Tobinai (National Cancer Center Hospital, Tokyo).

All of the eight eligible cases were diagnosed as smoldering ATL. Macroscopic findings were nodulotumoral in six cases, a subcutaneous tumor in one case and plaque in one case, and the number and size were heterogeneous in each case. Pathological findings of all eight cases were consistent with T-cell lymphoma, high-grade type (pleomorphic, medium or large size), with prominent perivascular infiltration and scant epidermotropism. Median times from the diagnosis to acute crisis, and onset of the cutaneous lesion to acute crisis, were 6 and 7 months, respectively (data not shown).

## DISCUSSION (PROBLEMS AND TO-DO LIST)

Accurate evaluation is essential at onset: cutaneous lesion at onset, lymph nodes and other organ involvement using computed tomography (CT) and/or positron emission tomography (PET)/CT, as well as the percentage of abnormal lymphocytes in peripheral blood (PB).

The clinical course of each lesion including cutaneous lesions should be evaluated with respect to the timing of diagnosis. As for the "extranodal primary cutaneous variant", further case evaluation is essential, including those with a relatively favorable prognosis.

As for the pathological diagnosis of cutaneous lesions, the biopsy site including macroscopic findings should be described. It is possible that specimens were biopsied at sites with a poor prognostic hierarchy in this case series.

In general, pathological findings of cutaneous lesion of ATL appear to be epidermotropic and non-epidermotropic. All of the cases in this meeting were high-grade peripheral T-cell lymphoma (PTCL)-like, and no case was low-grade cutaneous T-cell lymphoma-like because cases with a poor prognosis were collected.

Seven out of eight eligible cases were the "extranodal primary cutaneous variant", consisting of six cases of nodulotu-

moral and one of plaque macroscopically, and all seven were high-grade PTCL microscopically. The remaining one was described as a "primary subcutaneous tumor". There was a comment against the "primary subcutaneous tumor" type included in the "primary cutaneous variant".

To diagnose such cases as the "lymphoma type of ATL, extranodal primary cutaneous variant", it is essential to examine each case carefully, including cutaneous lesions at onset, lymph nodes and other organ involvement using CT and/or PET/CT, as well as the percentage of abnormal lymphocytes in PB (Appendix 1).

The application of clinical staging based on the extension of cutaneous lesions requires further investigation.

Some ATL patients with multipapular type cutaneous lesions were reported to show a rapidly progressive clinical course.[6] Such cases should also be collected and analyzed.

## FUTURE PLAN

More cases should be collected and investigated.

Based on the results of an ongoing nationwide survey on ATL, ATL with cutaneous lesions will be analyzed to investigate the incidence and prognosis of the so-called the "lymphoma type of ATL, extranodal primary cutaneous variant".

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**CONFLICT OF INTEREST:** None.

## REFERENCES

1 Shimoyama M, Members of the Lymphoma Study Group (1984–87). Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: a report from the Lymphoma Study Group (1984–87). *Br J Haematol* 1991; **79**: 428–437.

2 Lymphoma Study Group (1984–87). Major prognostic factors of patients with adult T-cell leukemia-lymphoma: a cooperative study. *Leuk Res* 1991; **15**: 81–90.

3 Ohshima K, Jaffe ES, Kikuchi M. Adult T-cell leukemia/lymphoma, In: Swerdlow SH, Campo E, Harris NL, eds. *WHO Classification of Tumour of Haemaopoietic and Lymphoid Tissues*. 4th edn. Lyon: IARC Press, 2008; 281–284.

4 Bittencourt AL, da Graças Vieira M, Brites CR, Farre L, Barbosa HS. Adult T-cell leukemia/lymphoma in Bahia, Brazil: analysis of prognostic factors in a group of 70 patients. *Am J Clin Pathol* 2007; **128**: 875–882.

5 Amano M, Kurokawa M, Ogata K *et al.* New entity, definition and diagnostic criteria of cutaneous adult T-cell leukemia/lymphoma: human T-lymphotropic virus type 1 proviral DNA load can distinguish between cutaneous and smoldering types. *J Dermatol* 2008; **35**(5): 270–275.

6 Sawada Y, Hino R, Hama K *et al.* Type of skin eruption is an independent prognostic indicator for adult T-cell leukemia/lymphoma. *Blood* 2011; **117**(15): 3961–3967.

7 Katsuya H, Yamanaka T, Ishitsuka K *et al.* Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma. *J Clin Oncol* 2012; **30**(14): 1635–1640.

## APPENDIX I

For the diagnostic criteria of the lymphoma type of the extranodal primary cutaneous variant, no definite appearance of abnormal cells in PB (≦1%) is essential.

Evaluation of abnormal lymphocytes by flow cytometry as well as based on the morphology is warranted to calculate the cells as a real number. However, such criteria are quite different from the original criteria for the definition of ATL and clinical subtype classification of ATL. Therefore, such a proposal requires careful analyses and evaluation. Discussion on this issue is currently limited in this meeting.

For ATL, quantitative evaluation of cutaneous lesions such as using the modified Severity Weighted Assessment Tool should be investigated; however, it is not easily applicable.

Macroscopic findings of cutaneous lesions are a significant prognostic factor in ATL. However, the combination of other parameters, for example, tumor markers such as lactate dehydrogenase and soluble IL-2 receptor, needs to be investigated.

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REVIEW ARTICLE

# Current therapy of choice for cutaneous lymphomas: Complementary to the Japanese Dermatological Association/ Japanese Skin Cancer Society guidelines

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## ABSTRACT

**The first Japanese edition of guidelines for management of cutaneous lymphoma was published jointly in 2009 by the Japanese Dermatological Association (JDA) and the Japanese Skin Cancer Society (JSCS) – Lymphoma Study Group; the guidelines were revised in 2011, and published in English in 2013. The JDA/JSCS guidelines are unique because they describe information and management practices for lymphomas specific to Asia, such as adult T-cell leukemia/lymphoma and extranodal natural killer/T-cell lymphoma, nasal type. In the present article, we have highlighted the essential points of management for cutaneous lymphomas in Asia. In order to complement the guidelines, we have added further information and our clinical experience of some currently available agents for cutaneous lymphomas in Japan.**

**Key words: adult T-cell leukemia/lymphoma, extranodal natural killer/T-cell lymphoma, guideline, interferon-γ, mycosis fungoides, vorinostat.**

## INTRODUCTION

Types of lymphomas and their incidences may vary among geographic areas and ethnic groups. In a series of 1733 patients with cutaneous lymphomas registered in Japan between 2007 and 2011, 1485 (85.7%) patients had lymphomas with a mature T- or natural killer (NK)-cell phenotype, and 224 (12.9%) patients were diagnosed as B-cell lymphomas.[1] The remaining 24 (1.4%) patients had blastic plasmacytoid dendritic cell neoplasm. Of 1733 patients, mycosis fungoides (MF) was the most common subtype of cutaneous lymphomas (750 patients, 43.3%) and 73% of the MF patients were in the early stage (stage IA, IB and IIA) of the disease. The incidence rate of adult T-cell leukemia/lymphoma (ATLL) among cutaneous lymphomas has been estimated as 16.7% in Japan, but is much lower in other Asian countries such as mainland China and Korea.[1–3] The incidence of extranodal NK/T-cell lymphoma, nasal type (ENKL) was 2% among cutaneous lymphomas. Previous investigators have reported that ENKL accounts for 15% of all cases of non-Hodgkin's lymphoma in the southwest region of China, 6.1% in Korea, 2.8% in Taiwan and 2.6% in Japan.[2–6] Therefore, ATLL and ENKL are the subtypes that may account for the higher incidence of mature T- and NK-cell neoplasms in Asian countries compared with those in the USA and Europe.

The first Japanese edition of guidelines for the management of cutaneous lymphoma was published jointly in 2009 by the Japanese Dermatological Association (JDA) and the Japanese Skin Cancer Society (JSCS) – Lymphoma Study Group,[7] then revised in Japanese in 2011,[8] and published in English in 2013.[9] The JDA/JSCS guidelines refer to information and management of cutaneous lymphoma specific to Asian countries, such as ATLL and ENKL.

## MF AND SÉZARY SYNDROME (SS)

Mycosis fungoides is an indolent cutaneous lymphoma, which accounts for approximately 45% of primary cutaneous lymphomas. Because the clinical course may extend for 10 years or more, it is difficult to evaluate the success or failure of therapeutic intervention. So far, there have been fewer randomized studies on this entity than on other types of lymphomas.[10–14]

### The JDA/JSCS guidelines for MF/SS

The current JDA/JSCS guidelines for MF/SS[9] have described therapeutic modalities that have not yet been approved by the Japanese National Health Insurance system (Fig. 1) (Tables 1,2). After the therapeutic guidelines were launched, clinical studies have been conducted to prove the efficacy and safety of various agents, including an immunomodulator

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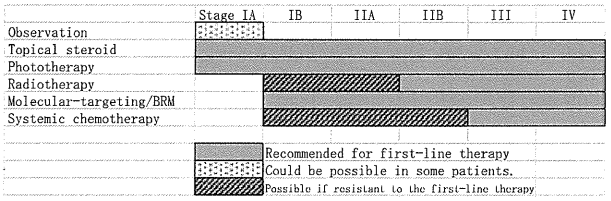


**Figure 1.** Therapy of choice for mycosis fungoides/Sézary syndrome.

**Table 1.** Japanese Dermatological Association/Japanese Skin Cancer Society guidelines for management of early stages of mycosis fungoides (MF)/Sézary syndrome

| Treatment | Degree of recommendation |
|---|---|
| First-line therapy recommended for early MF (stages I and IIA CTCL) | |
| Monitoring the clinical course without treatment | C1 (stage IA only)/C2 |
| Topical steroid therapy | B (for stage IA/IB) |
| ACNU topical therapy | C1 (small area, short term) |
| Broadband ultraviolet B | B (for stage IA/IB) |
| Narrowband ultraviolet B | B |
| PUVA | B |
| Localized radiation therapy† | B |
| Second-line therapy for early MF (resistant to the first-line treatments) | |
| TSEB‡,§ | B |
| Etretinate¶,†† | B–C1 |
| IFN-α¶,‡‡ | B–C1 |
| IFN-γ¶ | B–C1 |
| RePUVA¶,§§ | B |
| IFN-α + PUVA¶,‡‡ | B |
| IFN-γ + PUVA¶ | B |
| Chemotherapy¶¶ | D/B¶¶ |

Degree of recommendation classification: A, strongly recommended for implementation; B, recommended for implementation; B–C1, recommended for implementation, but less strongly supported than B; C1, implementation can be considered, but evidence* is insufficient; C2, no evidence*, cannot be recommended; and D, recommended not to implement. †For "minimal" stage IA unilesional mycosis fungoides, or where multiple lesions are localized within the same radiation field or multiple field in close proximity, and palliative radiation for infiltrated plaques resistant to topical therapy other than radiation. ‡Total skin electron beam (TSEB). §TSEB can be used as first-line therapy for stage IB/IIA (T2) with intense subjective symptoms accompanied by extensive highly infiltrated plaques and histopathological confirmation of folliculotropic mycosis fungoides or large cell transformation. ¶Can be a first-line treatment if systemic therapy is required (B1 or histopathological confirmation of folliculotropic mycosis fungoides or large cell transformation). BRM therapy (etretinate, interferon [IFN]-α, IFN-γ) can be used as monotherapy or in concomitant administration with psoralen plus ultraviolet A therapy (PUVA), and its concomitant use can also be investigated with topical therapies other than PUVA. ††Duration of response to oral etretinate is usually short; consider for use as concomitant therapy. ‡‡IFN-α therapy has been used in only a few cases in Japan. §§Retinoid-PUVA. ¶¶Third-line therapy for stage IB/IIA disease resistant to skin-targeted therapy and BRM therapy. ACNU, nimustine; CTCL, cutaneous T-cell lymphoma.

(lenalidomide), a proteasome inhibitor (bortezomib), histone deacetylase inhibitors (vorinostat, romidepsin and panobinostat), an antifolate (pralatrexate), biologics (alemtuzumab, siplizumab, denileukin diftitox and brentuximab vedotin) and nucleoside analogs (fludarabine, gemcitabine, nelarabine and forodesine).[15] The present article provides additional information on currently available agents for MF/SS in Japan, some of which have already been used widely in Western countries.

**Table 2.** Japanese Dermatological Association/Japanese Skin Cancer Society guidelines for management of MF/SS (stages IIB and III)

| Treatment | Degree of recommendation |
|---|---|
| First-line therapy recommended for stage IIB CTCL | |
| Etretinate | B–C1 |
| IFN-α†,‡,§ | B–C1 |
| IFN-γ†,§ | B–C1 |
| PUVA ± localized radiation therapy¶ | B |
| Localized radiation therapy¶ | B |
| TSEB†† | B |
| First-line therapy recommended for stage III CTCL | |
| ECP‡‡ ± IFN-α§§ | B |
| TSEB + ECP | B |
| Concomitant use of BRM and skin-directed therapy described above | B–C1 |

†Concomitant use of BRM (etretinate, interferon [IFN]-α, IFN-γ) and skin-directed therapy. ‡Concomitant therapy with IFN-α and psoralen plus ultraviolet A therapy (PUVA): degree of recommendation = B. §Can be used first-line therapy. ¶Palliative radiation for localized tumors. ††If lesion extend over <10% of body surface area. ‡‡Extracorporeal photochemotherapy. §§Total skin electron beam (TSEB) monotherapy can be used as first-line therapy for stage IIIA disease. CTCL, cutaneous T-cell lymphoma; ECP, extracorporeal photopheresis.

## Vorinostat for MF/SS

Vorinostat is a histone deacetylase inhibitor, with antineoplastic effects via induction of tumor suppressor genes and apoptosis. A previous phase II study of vorinostat for cutaneous T-cell lymphoma (CTCL) showed an objective response rate of approximately 30% in patients with stage IIB or more advanced disease.[16] A phase I study of vorinostat (400 mg/day, p.o.) was conducted to evaluate the safety, tolerability, pharmacokinetics and efficacy in six Japanese patients with relapsed or refractory MF.[17] In the phase I study, the most common drug-related adverse events were nausea (4/6, 67%), thrombocytopenia (4/6, 67%), hyperbilirubinemia (3/6, 50%) and vomiting (3/6, 50%). Of the six patients, an unconfirmed partial response was observed in one, and sustained stable disease for 12 weeks or longer was observed in two patients. After official approval of vorinostat for CTCL, more than 190 patients with MF/SS have been treated with vorinostat in Japan

In our series, we have experienced patients with thrombocytopenia, general fatigue, hyperglycemia, appetite loss, renal dysfunction, taste disorder and thrombosis, as previously pointed out.[16] Therefore, we should consider management to avoid such adverse reactions due to vorinostat. One of our patients with stage IVA2 presented with acute tumor and lymph node necrosis 2 days after intake of vorinostat 300 mg/day, associated with high-grade fever. The clinical and

histopathological findings suggest microthrombosis or infarction in the lesions, followed by induction of a host immune response (Fig. 2). Discontinuous use of vorinostat 200 mg/day, together with aspirin 100 mg/day was well-tolerated. Although vorinostat was tolerated at a dose of 400 mg/day in the phase I study,[17] the dose is reduced to 200–300 mg/day in many cases because of gastrointestinal symptoms and general fatigue.

It is intriguing to note that even in the same patients, some lesions responded to vorinostat, while others would not. We believe that a combination of skin-directed therapy with phototherapy, radiotherapy or chemotherapy should be considered for vorinostat-resistant lesions.

### Biological response modifier: Interferon-γ for MF/SS

Interferon (IFN)-α and IFN-γ were recommended as first-line therapy, in combination with skin-directed treatments, for patients with stages IIB and III MF/SS, and a second-line therapy for stages IA, IB to IIIB patients (Tables 1,2).[9] MF/SS are regarded as T-helper (Th)2-skewed disease characterized by eosinophilia, elevated serum levels of immunoglobulin E, interleukin (IL)-4 and IL-5,[18] and the expression of Th2-related CC chemokine ligand (CCL)11, CCL17 and CCL26.[19] Furthermore, our case with SS showed aggravation of the illness associated with a Th2-skewed immune reaction on staphylococcal infections, and improved with a Th1 shift on *Mycobacterium avium* infection.[20] It is, therefore, convincing that biological response modifiers such as IFN-α and IFN-γ have previously been used for treatments of MF/SS, in combination with skin-directed therapy.[21] Unfortunately, previously marketed IFN-γ products have not been available for treatment of MF/SS and ATLL since 2010. We conducted a multicenter, open-label, non-randomized, single-arm phase II study to evaluate the efficacy and safety of IFN-γ (Imunomax-γ; Shionogi, Osaka, Japan) for patients with stage IA to IIIA MF.[22] Of 15 patients who received 2 million Japan reference units of IFN-γ, once a day, over 5 days for 4 weeks, an objective response was observed in 11 patients (73.3%) by the response criteria of Ishihara *et al.*,[23] and in nine patients (60.0%) by the modified severity weighted assessment tool.[24] Influenza-like symptoms occurred in all patients, but such adverse reactions were tolerable. One patient died of aggravation of MF 50 days after the initiation of the study protocol. The phase II study proved that IFN-γ therapy was effective and tolerable in the management of patients with MF.

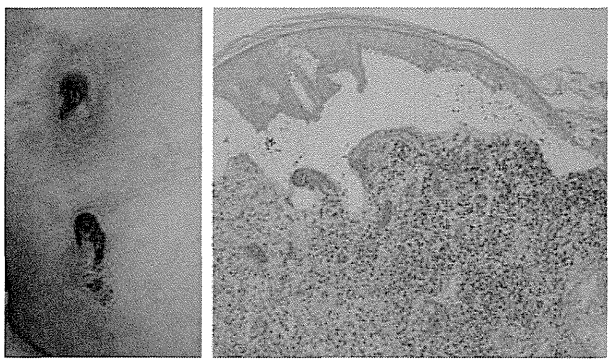

**Figure 2.** Acute tumor necrosis associated with thrombosis after intake of vorinostat.

### Bexarotene for MF/SS

Bexarotene is a synthetic retinoid analog named "rexinoid" that specifically activates retinoid X receptors. Bexarotene has been approved in the USA and Europe for the treatment of refractory CTCL. Bexarotene therapy is generally initiated at the lower dose of 150 mg/m$^2$ per day for 2–4 weeks, then titrated up to an optimal full dose of 300 mg/m$^2$ per day.[25–29] Topical bexarotene therapy has also been reported as having benefits for refractory or persistent early-stage MF.[30] Overall response rates were 54% in early stage MF (stages IA–IIA) and 45% in advanced stage MF (stage IIB–IVB).[25,26] Prescribing physicians must monitor hyperglycemia, especially triglyceridemia and central hypothyroidism due to the decreased secretion of thyroid-stimulating hormone. Bexarotene is contraindicated in pregnancy. Clinical studies of oral bexarotene for management of CTCL in Japan are close to being completed.

### Gemcitabine for MF/SS

Gemcitabine (2′,2′-difluorodeoxycytidine) is a pyrimidine analog which has been used for treatment of leukemias and lymphomas including CTCL.[31–34] Overall response rates to gemcitabine have ranged 62.5–75% for CTCL patients, depending on the administration doses, clinical stages of patients and pretreatments. Adverse reactions have included bone marrow suppression, hemolytic uremic syndrome, pulmonary embolism, hepatic damage, mucositis, infections, cardiac failure, influenza-like symptoms and skin rashes.

### Combination therapy for MF/SS

Various combination therapies are usually required for management of MF/SS because various types and T stages of skin lesions are intermingled in the same individuals. The usual combination may include retinoids plus phototherapy, retinoids plus IFN or phototherapy plus vorinostat (Table 1). One prospective, randomized clinical trial showed that IFN-α plus psoralen plus ultraviolet A therapy (PUVA) was superior to IFN-α plus acitretin in achieving complete remission in CTCL stages I and II.[10] Another report has proved the beneficial effects of PUVA plus IFN-α in achieving high remission rates and prolonging progression-free survival when compared with PUVA alone.[35] Combinations of conventional skin-directed treatments with vorinostat and gemcitabine have been under investigation.

## CUTANEOUS T/NK-CELL LYMPHOMA OTHER THAN MF/SS (NON-MF/SS)

Non-MF/SS cutaneous lymphomas are classified into two broad categories: (i) relatively aggressive lymphomas with poor prognosis (aggressive group); and (ii) indolent lymphomas with favorable prognosis (indolent group). The former group includes primary cutaneous CD8-positive aggressive epidermotropic

cytotoxic T-cell lymphoma, primary cutaneous γδ T-cell lymphoma, and peripheral T-cell lymphoma, not otherwise specified. It is, however, difficult to predict the prognosis of individual patients by cytological and immunophenotypic findings, without observation periods. For patients without general symptoms or notable laboratory test findings, skin-directed therapies used for MF/SS may be chosen as a first-line treatment.

An algorithm has been prepared for patients with indolent cutaneous lymphomas, which includes primary cutaneous anaplastic large cell lymphoma (pcALCL), subcutaneous panniculitis-like T-cell lymphoma and primary cutaneous CD4-positive small/medium T-cell lymphoma.[9] Because patients with pcALCL may take a progressive clinical course with regional lymph node involvement or disseminated skin lesions, polychemotherapy is sometimes required. Chimeric and humanized anti-CD30 monoclonal antibodies such as brentuximab (SGN-30) and MDX-060, both showed low overall response rates.[36,37] Brentuximab vedotin (SGN-35) is a conjugate of antitubulin agent monomethyl auristatin E and CD30-specific monoclonal antibody, which was designed to enhance antitumor activity. In 2011, brentuximab vedotin was approved for treatment of relapsed CD30-positive lymphomas, with objective responses in 17 of 45 patients with refractory disease.[38]

## ATLL

Adult T-cell leukemia/lymphoma is a peripheral T-cell malignancy caused by human T-cell leukemia virus type 1 (HTLV-1) and occurs in certain areas where HTLV-1 infections are endemic, including Asia. Three major infection routes have been proven: (i) blood transfusion from HTLV-1 carriers; (ii) breast feeding; and (iii) sexual transmission, mainly from male to female. A sero-epidemiological survey by Tajima *et al.*[39] demonstrated that HTLV-1 infections are prevalent in Japanese, native Andeans, Iranians, Central Africans and those of African descent in the Caribbean Basin and South America. It is noteworthy that extremely low incidences of seropositivity and occurrence of ATLL were found in Korea and Eastern China, neighboring countries of Japan.

Shimoyama[40] classified ATLL into four subgroups based on hematological findings, blood chemistry results and organ involvements: acute, chronic, lymphomatous and smoldering types. More than 50% of ATLL patients present with cutaneous lesions including disseminated papules, nodules and tumors. Scaly erythemic plaques and erythroderma indistinguishable from those of MF/SS occur in some patients. Approximately 5% of HTLV-1 carriers may develop ATLL or HTLV-1-associated disorders in 50 years. In other words, the remaining 95% of HTLV-1 carriers do not experience any HTLV-1-related disorders throughout their lives even though they continue to harbor HTLV-1-infected T-cells.

### Initial treatment for ATLL

Treatments of choice for ATLL should be determined by the subtypes and patients' conditions. For patients with ATLL lesions limited to the skin, PUVA, radiotherapy, oral retinoids, monochemotherapy using etoposide or a combination of these may be useful although beneficial effects on the prognosis of patients have not been confirmed.[9]

Recommended polychemotherapy for acute and lymphomatous types includes vincristine, cyclophosphamide, doxorubicin and prednisolone (VCAP), doxorubicin, ranimustine and prednisolone (AMP), and vincristine, etoposide, carboplatin and prednisolone (VEMP) regimens. The VCAP-AMP-VEMP (modified LSG15) regimen may be superior to biweekly CHOP, but the median survival time of 13 months still compares unfavorably to other T-cell malignancies.[41] A combination treatment with IFN-α and zidovudine may result in favorable response rates, particularly in acute, chronic and smoldering types of ATL.[41] Allo-hematopoietic stem cell transplant (HSCT) is a possible option for young patients with aggressive ATLL, but it remains to be answered which protocol of allo-HSCT is suitable for ATLL. Biologics have been developed for treatment of ATLL because of the unique immunophenotypic expression of CD2, CD25, CD52 and the Th2 type chemokine receptor, CCR4.

### Mogamulizumab for refractory ATLL

Mogamulizumab is an humanized anti-CCR4 monoclonal antibody with a defucosylated Fc region, which markedly enhances antibody-dependent cellular cytotoxicity. CCR4 is known to be expressed on regulatory T cells, Th2 cells and ATLL cells. Therefore, a multicenter phase II study was conducted to assess the efficacy, pharmacokinetic profile and safety in patients with relapsed CCR4-positive aggressive ATLL.[42] The patients received eight weekly i.v. infusions of mogamulizumab 1.0 mg/kg. Objective responses were observed in 13 (50%) of 26 patients (95% confidence interval, 30–70%) (Fig. 3). The most common adverse events were infusion reactions and skin rashes. Duvic *et al.*[43] recently reported a phase I/II study of mogamulizumab for refractory CTCL with or without CCR4 expression, showing an overall response rate of 39%.

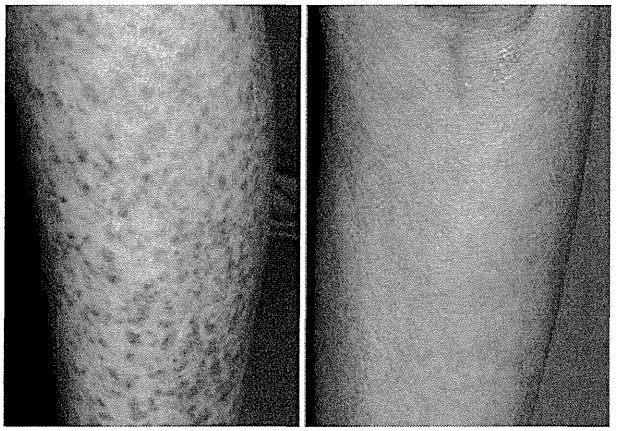

**Figure 3.** A patient with adult T-cell leukemia/lymphoma before and after mogamulizumab treatment.

## ENKL

In addition to a prototype of Epstein–Barr virus (EBV)-associated NK/T-cell lymphomas, ENKL, hydroa vacciniforme (HV)-like lymphoma has been listed in the World Health Organization (WHO) classification 2008 (Fig. 4). As reported by Lee and Ko,[44] both ENKL and HV-like lymphoma have been reported in Mexico, Peru and Asia, including Japan. The vast majority of ENKL cases are caused by EBV-infected NK cells, whereas HV-like lymphoma is a form of EBV-associated T-cell lymphoma. HV-like lymphomas occur most frequently in children and adolescents, and are often accompanied by hypersensitivity to mosquito bites and hemophagocytic syndrome (HPS). The prognosis of patients with classical HV is usually favorable, but one-third of patients with systemic HV (synonymous for HV-like lymphoma) died of HPS and multi-organ failure 10 years after onset (Miyake T, Yamamoto T, Hirai Y, Otsuka M, Hamada T, Morizane S, Iwatsuki K, unpubl. data). Univariate analysis revealed two poor prognostic indicators in such cases: (i) onset age over 9 years; and (ii) the expression of an EBV reactivation signal, BZLF1, in the skin lesions.

Radiation therapy with a simultaneous or subsequent DeVIC regimen (dexamethasone, VP16, ifosfamide, carboplatin) is recommended for localized lesions, and the SMILE regimen (dexamethasone, methotrexate, ifosfamide, L-asparaginase and etoposide) for generalized lesions of ENKL.[45] For HV-like lymphoma, polychemotherapy followed by HSCT has been chosen in many cases,[46] but HSCT-related complications and death occasionally occur.

## CUTANEOUS B-CELL LYMPHOMA

The WHO 2008 classification of hematopoietic malignancies has listed the nomenclature of cutaneous B-cell lymphomas as follows: extranodular marginal zone B-cell lymphoma (cMZL); primary cutaneous follicle center cell lymphoma (PCFCL), primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, leg type); PCLBCL, not otherwise specified; and intravascular large B-cell lymphoma (IVL). Disease type is an important prognostic factor for cutaneous B-cell lymphoma. Both PCFCL and MALT-type lymphomas are indolent-type lymphomas with a favorable prognosis, while prognosis is poor in PCLBCL and IVL. In 2008, the European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphomas released guidelines for the treatments of cutaneous B-cell lymphoma, based on previous reports.[47] Most of the reported treatment methods for topical therapy involved radiation and/or surgical resection. Radiotherapy or surgical resection is recommended for diseases in the indolent group (cMZL and PCFCL).

Most of the methods for systemic therapy involve chemotherapy and the administration of rituximab.[48] Rituximab may be useful for the treatment of diseases in the indolent group (cMZL and PCFCL), particularly in cases of multiple lesions. One should consider that $CD20^-$ $CD138^+$ plasmacytoid neoplastic cells are resistant to rituximab monotherapy. Combination chemotherapy may be considered for diseases in the indolent group that are refractory to other treatment regimens and for advanced extracutaneous disease. Combination chemotherapy, and particularly the concomitant use of rituximab, is recommended for PCLBCL, leg type, and for IVL, but rituximab monotherapy is also possible for the treatment of PCLBCL in cases where combination therapy may be poorly tolerated, such as in the elderly and in patients with severe complications.

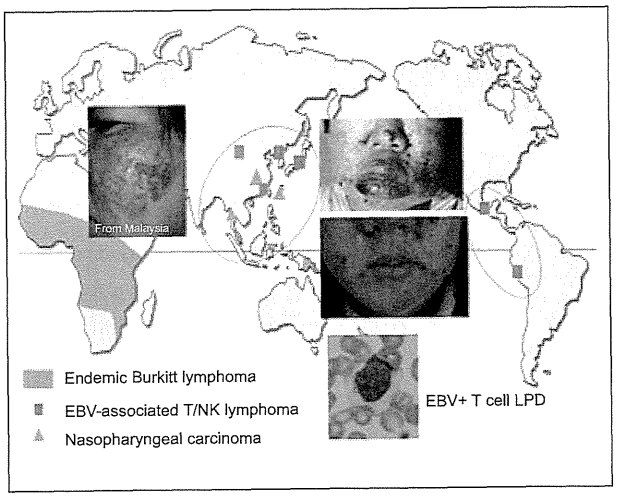


**Figure 4.** Hydroa vacciniforme-like lymphoma (synonymous with systemic hydroa vacciniforme) in Asia.

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## REFERENCES

1 Hamada T, Iwatsuki K. Cutaneous lymphomas in Japan: a nationwide study of 1,733 patients. *J Dermatol* 2014; **41**: 3–10.
2 Lee MY, Tan TD, Feng AC *et al.* Clinicopathological analysis of malignant lymphoma in Taiwan, defined according to the World Health Organization classification. *Haematologica* 2005; **90**: 1703–1705.
3 Lymphoma Study Group of Japanese Pathologists. The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. *Pathol Int* 2000; **50**: 696–702.

4 Ko YH, Kim H, Oh Y *et al.* EBV-associated T and NK cell lymphoproliferative disorders: consensus report of the 4th Asian Hematopathology Workshop. *J Hematopathol* 2012; **5**: 319–324.
5 Kim JM, Ko YH, Lee SS *et al.* WHO Classification of malignant lymphomas in Korea: report of the third nationwide study. *Korean J Pathol* 2011; **45**: 254–260.
6 Yang QP, Zhang WY, Yu JB *et al.* Subtype distribution of lymphomas in southwest China: analysis of 6382 cases using WHO classification in a single institution. *Diagn Pathol* 2011; **6**: 77.
7 Iwatsuki K, Kawai K, Ohtsuka M *et al.* Guidelines for the management of cutaneous malignant tumors II: cutaneous lymphomas. *Jap J Dermatol* 2009; **119**: 1189–1211.
8 Sugaya M. Guidelines for the management of cutaneous lymphomas (2011). *Jap J Dermatol* 2012; **122**: 1513–1531.
9 Sugaya M, Hamada T, Kawai K *et al.* Guidelines for the management of cutaneous lymphomas (2011): a consensus statement by the Japanese Skin Cancer Society – Lymphoma Study Group. *J Dermatol* 2013; **40**: 2–14.
10 Stadler R, Otte HG, Luger T *et al.* Prospective randomized multicenter clinical trial on the use of interferon-2a plus acitretin versus interferon-2a plus PUVA in patients with cutaneous T-cell lymphoma stages I and II. *Blood* 1998; **92**: 3578–3581.
11 Stadler R, Kremer A, Luger T, Sterry W. Prospective, randomized, multicentre clinical trial on the use of interferon a 2a plus PUVA versus PUVA monotehrapy in patients with cutaneous T-cell lymphoma, stage I and II. *J Clin Oncol* 2006 ASCO Annual Meeting Proceedings 2006; **24**: 7541.
12 Kaye FJ, Bunn PA Jr, Steinberg SM *et al.* A randomized trial comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. *N Engl J Med* 1989; **321**: 1784–1790.
13 Child FJ, Mitchell TJ, Whittaker SJ *et al.* A randomized cross-over study to compare PUVA and extracorporeal photopheresis in the treatment of plaque stage (T2) mycosis fungoides. *Clin Exp Dermatol* 2004; **29**: 231–236.
14 Prince HM, Duvic M, Martin A *et al.* Phase III placebo-controlled trial of denileukin diftitox for patients with cutaneous T-cell lymphoma. *J Clin Oncol* 2010; **28**: 1870–1877.
15 Foss FM, Zinzari PL, Vose JM *et al.* Peripheral T-cell lymphoma. *Blood* 2011; **117**: 6756–6767.
16 Olsen EA, Kim YH, Kuzel TM *et al.* Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2007; **25**: 3109–3115.
17 Wada H, Tsuboi R, Kato Y *et al.* Phase 1 and pharmacokinetic study of the oral histone deacetylase inhibitor vorinostat in Japanese patients with relapsed or refractory cutaneous T-cell lymphoma. *J Dermatol* 2012; **39**: 823–828.
18 Dummer R, Heald PW, Nestle FO *et al.* Sézary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon-gamma receptor beta-chain). *Blood* 1996; **88**: 1383–1389.
19 Kakinuma T, Sugaya M, Kaneko F *et al.* Thymus and activation-regulated chemokine (TARC/CCL17) in mycosis fungoides: serum TARC levels reflect the disease activity of mycosis fungoides. *J Am Acad Dermatol* 2003; **48**: 23–30.
20 Yamada A, Yamasaki O, Asagoe K *et al.* Recovery from Sezary syndrome following *Mycobacterium avium* spondylitis. *Br J Dermatol* 2007; **157**: 1270–1271.
21 Whittaker SJ, Foss FM. Efficacy and tolerability of currently available therapies for the mycosis fungoides and Sezary syndrome variants of cutaneous T-cell lymphoma. *Cancer Treat Rev* 2007; **33**: 146–160.
22 Sugaya M, Tokura Y, Hamada T *et al.* Phase II study of intravenous interferon-g in Japanese patients with mycosis fungoides. *J Dermatol* 2014; **41**: 50–56.
23 Ishihara K. Late phase II clinical study of OH-6000 for adult T cell leukemia and lymphoma. *Skin Cancer* 1993; **8**: 352–367.
24 Olsen EA, Whittaker S, Kim YH *et al.* Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. *J Clin Oncol* 2011; **29**: 2598–2607.
25 Duvic M, Martin AG, Kim Y *et al.* Phase 2 and 3 clinical trial of oral bexarotene (Targretin capsules) for the treatment of refractory or persistent early-stage cutaneous T-cell lymphoma. *Arch Dermatol* 2001; **137**: 581–593.
26 Duvic M, Hymes K, Heald P *et al.* Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II–III trial results. *J Clin Oncol* 2001; **19**: 2456–2471.
27 Gniadecki R, Assaf C, Bagot M *et al.* The optimal use of bexarotene in cutaneous T-cell lymphoma. *Br J Dermatol* 2007; **157**: 433–440.
28 Querfeld C, Rosen ST, Guitart J *et al.* Comparison of selective retinoic acid receptor- and retinoic X receptor-mediated efficacy, tolerance, and survival in cutaneous t-cell lymphoma. *J Am Acad Dermatol* 2004; **51**: 25–32.
29 Breneman D, Duvic M, Kuzel T *et al.* Phase 1 and 2 trial of bexarotene gel for skin-directed treatment of patients with cutaneous T-cell lymphoma. *Arch Dermatol* 2002; **138**: 325–332.
30 Heald P, Mehlmauer M, Martin AG *et al.* Topical bexarotene therapy for patients with refractory or persistent early-stage cutaneous T-cell lymphoma: results of the phase III clinical trial. *J Am Acad Dermatol* 2003; **49**: 801–815.
31 Jidar K, Ingen-Housz-Oro S, Beylot-Barry M *et al.* Gemcitabine treatment in cutaneous T-cell lymphoma: a multicenter study of 23 cases. *Br J Dermatol* 2009; **161**: 660–663.
32 Duvic M, Talpur R, Wen S *et al.* Phase II evaluation of gemcitabine monotherapy for cutaneous T-cell lymphoma. *Clin Lymphoma Myeloma* 2006; **7**: 51–58.
33 Zinzani PL, Baliva G, Magagnoli M *et al.* Gemcitabine treatment in pretreated cutaneous T-cell lymphoma: experience in 44 patients. *J Clin Oncol* 2000; **13**: 2603–2606.
34 Marchi E, Alinari L, Tani M *et al.* Gemcitabine as frontline treatment for cutaneous T-cell lymphoma: phase II study of 32 patients. *Cancer* 2005; **104**: 2437–2441.
35 Roenigk HH Jr, Kuzel TM, Skouteils AP *et al.* Photochemotherapy alone or combined with interferon-2a in the treatment of cutaneous T-cell lymphoma. *J Invest Dermatol* 1990; **95** (6 Suppl): 198S–205S.
36 Forero-Torres A, Leonard JP, Younes A *et al.* A phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphomas. *Br J Haematol* 2009; **146**: 171–179.
37 Ansell SM, Horwitz SM, Engert A *et al.* Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. *J Clin Oncol* 2007; **25**: 2764–2769.
38 Younges A, Barlett N, Leonard JP *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N Engl J Med* 2010; **363**: 1812–1821.
39 Tajima K, Takezaki T. Ethnoepidemiologicay of ATL/HTLV in Mongoloids with special reference to Mongoloid dispersal. *Gann Monogr Cancer Res* 2003; **50**: 277–288.
40 Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. A report from the Lymphoma Study Group (1984–87). *Br J Haematol* 1911; **79**: 428–437.
41 Tsukasaki K, Tobinai K. Clinical trials and treatment of ATL. *Leuk Res Treatment* 2012; **2012**: 101754.
42 Ishida T, Joh T, Uike N *et al.* Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter pahse II study. *J Clin Oncol* 2012; **30**: 837–842.
43 Duvic M, Pinter-Brown L, Foss FM *et al.* Results of a phase 1/2 study for KW-0761, a monoclonal antibody directed against CC chemokine receptor type 4 (CCR4), in CTCL patients. ASH Annual Meeting Abstracts 2010 116: abstract 962, Clincal trials. Gov: NCT00888927), Orlando, USA, 2010.
44 Park S, Ko YH. Epstein-Barr virus-associated T/Natural killer (NK) cell lymphoproliferative disorders. *J Dermatol* 2014; **41**: 29–39.

45 Yamaguchi M, Kwong YL, Kim WS *et al.* Phase II study of SMILE chemotherapy for newly diagnosed stage IV, relapsed, or refractory extranodal natural killer (NK)/T-cell lymphoma, nasal type: the NK-cell Tumor Study Group study. *J Clin Oncol* 2011; **33**: 4410–4416.
46 Kawa K, Sawada A, Sato M *et al.* Excellent outcome of allogeneic hematopoietic SCT with reduced-intensity conditioning for the treatment of chronic active EBV infection. *Bone Marrow Transplant* 2011; **46**: 77–83.
47 Sneff NJ, Noordijk EM, Kim YH *et al.* European Organization for Research and Treatment of Cancer and International. Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. *Blood* 2008; **112**: 1600–1609.
48 Suárez AL, Querfeld C, Horwitz S, Pulitzer M, Moskowitz A, Myskowski PL. Primary cutaneous B-cell lymphomas: part II. Therapy and future directions. *J Am Acad Dermatol* 2013; **69**: 343.e1–343.e11.

 

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# RELEVANCE OF MOLECULAR TESTS FOR HTLV-1 INFECTION AS CONFIRMATORY TESTS AFTER THE FIRST SERO-SCREENING

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□ *The diagnosis of human T-cell leukemia virus type-1 (HTLV-1) infection has been widely examined by serologics. In the first screening tests, serological false negative and positive samples have been reduced thanks to advances in assay techniques that apply new emission agents and sensors. On the other hand, western blot (WB) remains problematic. For example, WB analysis yields many samples equivalent to antibody positive ones. To reduce the need for WB, an alternative testing strategy is required to detect HTLV-1 infection. Polymerase chain reaction (PCR) for the HTLV-1 provirus has recently been recommended for a final diagnosis of infection. However, although PCR is thought to be one element, the validation of detection performance for HTLV-1 infection between serological and molecular testing is not always clear. Thus, this study aimed to evaluate the accuracy and test the validity of an improved methodology for serological detection of HTLV-infection, as well as that of PCR. In conclusion, the high values of kappa-statistics are expected to deliver high quality in chemiluminescent enzyme immunoassay (or chemiluminescent immunoassay), while the problems with WB assays remain to be elucidated. As an alternative to WB, a combination of real-time qPCR and nested PCR is proposed as a suitable confirmatory test.*

**Keywords** CLEIA, CLIA, HTLV-1, PCR, westernblot

## INTRODUCTION

Recently, the diagnosis of human T-cell leukemia virus type-1 (HTLV-1) infection has been widely examined by serological tests.[1–7] Screening of serum by particle agglutinations (PA) and chemi-luminescent immune assays (Chemiluminescent Enzyme Immunoassay: CLEIA and chemiluminescent immunoassay: CLIA) and confirmation by western blot (WB) is a common testing strategy.[8,9] However, when this strategy is applied to low infection prevalence populations, false positive samples may increase markedly, because the first screening tests are usually highly sensitive and

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low specific. Furthermore, WB as a confirmatory test is known to yield many indeterminate results. In the first screening tests, serological false negative and positive samples have been reduced thanks to advances in assay techniques applying new emission agents and sensors. On the other hand, WB remains problematic. For example, WB analysis yields many samples equivalent to antibody positive ones. To reduce the need for WB, an alternative testing strategy is required to detect HTLV-1 infection.

Polymerase chain reaction (PCR) for the HTLV-1 provirus has recently been recommended for a final diagnosis of infection.[10] However, although PCR is thought to be one element, the validation of detection performance for HTLV-1 infection between serological and molecular testing is not always clear. Thus, this article aimed to evaluate the accuracy and test the validity of an improved methodology for serological detection of HTLV-infection, as well as that of PCR.

## MATERIAL AND METHODS

### Materials

A total of 105 pregnant blood samples collected from January, 2011, to December, 2011, were used. During this period, 9,718 samples were tested for screening. 105 samples excluding double negative in PA and CLEIA samples were used as the secondary samples, and were examined for infection by 4 methods as described below. Meanwhile, using 25 practical blood samples collected from hospitalized patients in complete remission from adult T-cell leukemia (ATL), the role of PCR in serological detection of HTLV-1 infection was investigated.

Next, we examined low titer samples or sero-negative converted samples selected from 350 ATL patients who underwent bone marrow transplantation (BMT) and/or chemotherapy.

## METHODS

Serological detection of HTLV-1 infection was done using commercially available assay kits according to the manufacturer's instructions, with Serodia-HTLV-I (PA; Fuji-Rebio, Tokyo), Lumipulse-HTLV-I (CLEIA; Fuji-Rebio, Tokyo, Japan), Architect-HTLV (CLIA; Abbott, Chicago, IL, USA), and problot-HTLV-I (WB; Fuji-Rebio, Tokyo, Japan). The positivity of WB analysis was decided according to the WHO criteria.[9]

### HTLV-1 Proviral Load (PVL)

After separation of peripheral blood mononuclear cells (PBMC) in the Conray manner, genomic DNA was extracted using Qiagen kits (Qiagen,